INTERRELATIONSHIPS OF CERTAIN THERMAL AND ENDOCRINE PHENOMENA AND REPRODUCTIVE FUNCTION IN THE FEMALE BOVINE

BY

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Ten normally cycling Holstein heifers were assigned to one of two environmental treatment groups (21.3 C, 59% RH or 32.0 C, 67% RH). $PGF_{2\alpha}$ was used to cause luteal regression and synchronize estrus. Least-squares analyses were conducted to characterize treatment, animal and within-animal time trends in plasma progestins, estradiol, estrone, LH, prolactin and corticoids.

Environmental treatment (32.0 C) evoked a 1.49 C increase in rectal temperature and a 3.59 C increase in skin temperatures. Length of estrus was shorter ($P<.10$) for the 32.0 C heifers. Two of four heifers at 21.3 C inseminated were pregnant at 40 days compared to none of five at 32.0 C.

Average progestin concentration between treatments were not different ($P>.10$; .63 ng/ml at 21.3 C compared to .65 ng/ml at 32.0 C). Mean estradiol concentrations were significantly ($P<.10$) lower in 32.0 C heifers (3.45 pg/ml compared to 2.96 pg/ml). There was a significant elevation ($P<.05$) of estrone due to heat stress (1.55 pg/ml compared to 1.85). No significant differences ($P>.10$) were found in mean LH concentrations between heifers at 21.3 C or 32.0 C. Preovulatory peak LH

concentrations were 32.2 and 33.2 ng/ml plasma, respectively. All animals had a preovulatory LH surge, suggesting that hyperthermia did not prevent the triggering mechanism for LH release. Mean prolactin (14.51 ng/ml at 21.3 C compared to 14.78 ng/ml at 32.0 C) and corticoid (8.01 ng/ml at 21.3 C compared to 7.76 ng/ml at 32.0 C) concentrations were not different between temperature treatments ($P>.10$).

In an attempt to determine if plasma dilution may have occurred, total protein concentration and osmolality were measured. There was no difference ($P>.10$) in total protein concentration or osmolality between treatment groups. The affinity (K_a) of cortisol for CBG was not different between treatments ($P>.10$); however, the binding capacity of CBG for cortisol was reduced ($P<.05$) in the 32.0 C heifers.

Results of this experiment showed only subtle thermal effects on estradiol and estrone plasma concentrations and no effects on LH, progestins, corticoids and prolactin. Apart from possible hormonal involvement with duration of estrus, heat stress did not appear to affect the hormonal mileau in peripheral plasma associated with corpus luteum regression, follicle growth and ovulation.

Eight days following ovulation in the last heifer, 200 IU ACTH was injected, IV, into the 10 heifers. The 32.0 C heifers responded with significantly lower ($P<.10$) corticoid concentrations. The 6th order regression response curves were not parallel ($P<.01$) suggesting that the hot group response was earlier to reach a peak (75 compared to 105 min.), had a lower magnitude (73.5 compared to 100.2 ng/ml corticoids) and was of shorter duration (4 compared to 5 hr.).

Because the first experiment did not specifically consider environmental and hormonal effects on uterine temperature it was necessary to

document possible estrogen induced uterine thermal changes. In the second experiment thermocouples were placed into the uterine serosa and saphenous artery of four dairy heifers. Injection of 3 mg estradiol-17β caused a .25 C decrease ($P<.01$) in the difference between uterine and aortic temperature (ΔT_{u-a}) by 2.5 hr. postinjection. In contrast, there was no significant change ($P>.10$) in the ΔT_{u-a} after injection of saline. The final experiment was an attempt to document and evaluate changes in uterine temperature during the period of luteal regression, follicle growth and ovulation induced by $PGF_{2\alpha}$ under conditions of a mild heat stress. Thermocouples were placed into the uterine serosa and aortic blood vessel of four dairy cattle. $PGF_{2\alpha}$ caused an immediate drop in uterine and aortic temperatures, and a decrease in the ΔT_{u-a} of almost .4 C at 45 min. postinjection. The two cows, in which thermocouples remained operational for the duration of the study, had monophasic daily uterine and aortic temperature rhythms. However, both temperatures lagged about 6 hr. behind air temperature changes. Uterine temperatures reached 40 C for periods of up to 6 hr. Failure to detect an association between ΔT_{u-a} and hormonal measurements may have been due to a time lag association. Not until the preovulatory surge of LH was there an appreciable rise in ΔT_{u-a} ($P<.01$), and this occurred at a time when estradiol was decreasing. The mild environmental heat stress may have contributed to the high uterine and aortic blood temperatures.

William W Hatcher

Chairman

INTRODUCTION

Reduced reproductive efficiency occurs during the hot seasons of the year in many parts of the United States. Lowered conception rate due to heat stress occurs over a prolonged period of the year in Florida and represents a major production problem to dairymen. A 12 year study in the University of Florida dairy herd revealed a conception rate per service of less than 40% (Gwazdauskas, Thatcher and Wilcox, 1975). Economically, poor reproductive performance under conditions of thermal stress decreases heifer replacement availability and long term milk production and increases calving interval and culling rate.

Before systems for reproductive management can be developed to counter these adverse effects of heat stress, several fundamental questions must be answered. Among these are:

1. How does a standard heat stress alter hormonal and physiological responses during the normal estrous cycle? The objective of the first experiment (Section II) was to characterize hormonal changes (progestins, estradiol, estrone, LH, prolactin and corticoids), rectal temperature, plasma protein concentration and osmolality and plasma cortisol binding capacity (CBC) in heifers subjected to a standard heat stress (21.3 compared to 32.0 C). In addition, plasma

corticoids in response to ACTH were measured to evaluate possible thermal stress effects on adrenal responsiveness.

2. What are the factors influencing uterine temperature? Can estradiol, which is known to have a marked effect on uterine blood flow and metabolism, alter uterine temperature? What are the changes in uterine temperature during the period of luteal regression, follicle growth and ovulation under conditions of mild heat stress? In Section III a series of experiments were designed in an attempt to answer these questions.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes luteal regression in the bovine and has enabled the researcher to use it efficiently to control endocrine and physiological changes near the time of estrus and ovulation. Such a compound maximizes use of experimental facilities over short periods of time without adverse chronic alterations of normal bovine physiology. In answering questions one and two above, $PGF_{2\alpha}$ was used to synchronize the hormonal events associated with corpus luteum regression, estrus and ovulation.

SECTION I

REVIEW OF LITERATURE

Stress is defined as a condition harmful to an organism, which results from inability of the organism to maintain a constant internal environment (Taber, 1961). Factors involved in altering homeostasis include trauma, surgical operations, restraint, extreme cold or heat, intense solar radiation, social stress due to peck order, nutritional stress and internal stress caused by pathogens or toxins (Hafez, 1968; Guyton, 1966). The purpose of the initial review section is to report on the effects of thermal stress on reproductive performance with major emphasis on hormonal or endocrine aspects.

Influence of Thermal Stress on Reproductive Performance

Hot environments may exert their depressive effect on fertility via the gonads, accessory sex glands, uterine environment, gametes or endocrine system (Hafez, 1959; Ulberg and Burfening, 1967). Reproductive behavior has been shown to be altered by heat stress, in that estrous duration was shorter (Branton et al., 1957; Gangwar, Branton and Evans, 1965; Hall et al., 1959), there was an increased frequency of quiet ovulations (Labhsetwar et al., 1963) and anestrus (Bond and McDowell, 1972) and a reduction in estrous intensity (Gangwar, Branton and Evans, 1965).

High temperatures exert direct effects on fertilized ova grown in vitro (Alliston et al., 1965). Fertilized ova grown through first cell division at 40 C in vitro had a lower rate of embryo survival than those grown at 38 C when returned to synchronized pseudopregnant recipient rabbits. There were no morphological differences between ova in different media. As the period of culture at 40 C was delayed to second cell division, differences in post-implantation death losses disappeared. An environment of 32.2 C and 65% Relative Humidity (RH) did not inhibit estrus or alter ovulation rate in sheep (Alliston and Ulberg, 1961). However, an increase in embryo death was detected when embryos (2-32 cell stage) were transferred from donor ewes kept at 32.2 C to recipient ewes at 21.1 C ambient temperature. Embryo survival was highest when both donor and recipient ewes were maintained at 21.1 C, indicating that damage to the early embryo was most likely to occur in uteri of ewes kept at 32.2 C. Heat stressing one or both parents of a mouse embryo affected the rate of thymidine, uridine and guanine incorporation into nucleic acids during pre-implantation development, which may lead to altered DNA and RNA synthesis and subsequent embryonic mortality (Sheean, Durrant and Ulberg, 1974).

Due to limitations of facilities and methodology, most investigations of the effects of heat stress on hormonal balance and their relationships to reproductive performance have monitored only one or two hormonal responses. Therefore pooling results from different laboratories by combining data from different animals within and between species can be misleading when an effort is made to develop a hypothesis on how thermal stress affects reproduction. Stott, Thomas and Glenn

(1967) found progesterone to be elevated on the day of estrus in thermally stressed cows. Heifers maintained at 32 C and 21 C for 72 hr. beginning at the onset of estrus had conception rates of 0 and 48%, respectively, according to a report by Dunlap and Vincent (1971). Associated with the decreased fertility was an elevated plasma progestin concentration of only .42 ng/ml plasma (Mills et al., 1972). In contrast, Stott and Wiersma (1973) reported depressed plasma progestin levels during chronic heat stress in the bovine.

Evidence of detrimental progesterone effects on embryo cleavage stages has been reported by Dickman (1970). Fertilized ova were transferred on day 4 of pregnancy to pseudopregnant rats which had been ovariectomized on day 2. When transfer was preceded by 2, 3, 4, 5 or 6 days of progesterone treatment in pseudopregnant rats, 49, 38, 13, 2.5 and 2% of the transferred morula developed into fetuses. However, when blastocysts were transferred, there was a 62.5% fetal survival. Overstimulation with progesterone apparently interfered with embryonic development. Johnsson et al. (1974) reported a 60 to 75% reduction in fertility in ewes receiving a single injection of progesterone on days 0, 1, 2 or 3 or daily injections on days 1 to 4. The progesterone given before day 4 may have affected embryo transport through the oviduct or directly altered it and therefore inhibited or abolished its ability to cope with the 'luteolysin' and prevent corpus luteum regression during pregnancy.

Progesterone, superimposed on estradiol administration in ewes, caused a prompt decrease in uterine blood flow (Greiss and Anderson, 1970). Extreme or prolonged limitation of blood flow to the vicinity

of the embryo can result in fetal death. Blockage of blood supply to the uterus one day post coitum in the mouse was most detrimental to implantation rate (Senger et al., 1967). These observations may have been the result of the uterine coagulation procedure since necrosis of the tissue was detected (F. W. Bazer, personal communication). However, coagulation of blood vessels to one uterine horn resulted in 51% fewer embryos migrating to the uterus by 4 days after mating in mice. At 10 days post mating there were fewer live fetuses on the coagulated side compared to the control side (57% compared to 73%). Therefore, reduced blood flow may be responsible for failure of embryo transport to the uterus and increased fetal death rate (Bazer, Ulberg and LeMunyan, 1969). Thus, if heat stress increased plasma progesterone levels, an altered blood flow may be a factor associated with reduced fertility.

Heat stress has been shown to cause elevated plasma corticoid levels in the bovine within 4 hr. of exposure which suggests increased adrenal activity (Christison et al., 1970). Other workers reported that plasma corticoids decreased during chronic heat stress in cattle (Alvarez and Johnson, 1973; Christison and Johnson, 1972; Rhynes and Ewing, 1973) and that corticoid turnover rates decreased (Christison and Johnson, 1972). However, chronic hyperthermia resulted in elevated epinephrine and norepinephrine, though corticoids were depressed, which suggested a decreased sensitivity to physiological actions of catecholamines (Alvarez and Johnson, 1973). Shayanfar (1973) compared adrenal responsiveness to adrenocorticotropin (ACTH) in cows exposed to ambient temperatures of greater or less than 21.1 C. At ambient temperatures above 21.1 C, plasma corticoid response to ACTH was slower,

peak levels were lower and the response was of shorter duration. Yousef and Johnson (1967) reported a 30 to 40% increase in heat production following injections of hydrocortisone acetate to cattle at 35 C ambient temperature. Thus, during prolonged thermal stress, depressed plasma corticoids and lowered adrenal responsiveness to ACTH may be indicative of altered adrenal function.

Madan and Johnson (1971) have reported that the preovulatory peak of plasma LH and basal plasma LH concentrations were lower in heifers maintained at 33.5 C and 55% RH compared to those at 18.2 C and 55% RH. These results support the hypothesis that thermal stress may alter secretion or metabolism of various hormones associated with reproductive function. However, Riggs, Alliston and Wilson (1974) detected a breed difference in response of the pre-ovulatory surge of LH to heat stress in gilts. Heat stressed pigs of the Duroc breed had no difference in magnitude of the preovulatory plasma LH surge compared to controls, whereas pigs of the Hampshire breed had a three to sixfold increase in the preovulatory peak of LH compared to their controls. It appears that species and breeds may, therefore, respond differently to thermal stress and that inferences among species and breeds must be reviewed with caution.

Koprowski and Tucker (1973), Schams and Reinhart (1974) and Thatcher (1974) found elevated peripheral plasma prolactin concentrations during the hot months of the year, suggesting that photoperiod and temperature modulate prolactin release. At a constant day length, calves exposed to 27 C had significantly higher plasma prolactin concentrations than those exposed to 10 C, whereas at 27 C prolactin levels

were only slightly higher than at 21 C (Wetteman and Tucker, 1974). However, Karg and Shams (1974) found a positive correlation between day length and basal prolactin concentrations in male and female cattle. Relkin (1972) demonstrated that changes in light:dark ratios for rats altered pituitary content and plasma concentrations of prolactin. It would appear that this question has yet to be resolved in cattle, i.e. whether photoperiod or temperature is the primary factor affecting plasma prolactin concentrations.

These various studies suggest that thermal stress may alter circulating plasma concentrations of certain pituitary, adrenal and ovarian hormones. Such excesses or deficiencies of these hormones may influence certain reproductive phenomena and account for lowered fertility.

Environmental factors play an important role in bovine fertility. Seasonal depressions in conception rate due to heat stress effects on the male can be eliminated through A.I. (artificial insemination) in which semen from bulls can be collected and frozen during cooler times of the year. Under these circumstances Stott (1961) still found a seasonal depression in breeding efficiency of cows which paralleled high climatic temperatures in Arizona and California. Thus, this experiment indicated that altered reproductive efficiency in the female was the major contributor to summer depression of fertility.

In Florida, a year-long study was conducted to relate climatic, rectal and uterine temperatures, plasma corticoid and progesterone concentrations, breed, service number, time of service, sire and age to conception rate (Gwazdauskas, Thatcher and Wilcox, 1973). Significant

effects due to environmental temperature on the day after insemination, rectal and uterine temperatures at insemination, sire and days post-partum were detected on conception rate. Deleting environmental temperature from the statistical model revealed significant effects of uterine temperature the day after insemination on fertility. Relationships between uterine temperatures at insemination or the day after insemination with fertility were intriguing. Uterine temperature the day after insemination appeared to be positively associated with environmental temperature on that day. Their inverse associations with fertility may reflect direct detrimental thermal effects on early cleavage and development of the embryo. In contrast, the association of uterine temperature at insemination with fertility might be related to certain physiological (uterine blood flow and vaginal thermal conductance) and hormonal changes occurring at estrus that may be associated with proper timing of insemination to achieve maximal fertility.

In a subsequent study with 12 years of data, effects on conception rate of age of cow, inseminator, service sire, month, year, breed and 21 climatological variables were evaluated (Gwazdauskas, Wilcox and Thatcher, 1975). Age, inseminator, sire and breed had significant effects on conception. Maximum temperature the day after insemination, rainfall the day of insemination, minimum temperature the day of insemination, solar radiation the day of insemination and minimum temperature the day after insemination were the five highest ranking climatological variables associated with fertility. The most potent environmental variable, maximum temperature the day after insemination, had a significant curvilinear relationship with fertility. As maximum

temperature increased from 21.1 to 35 C, conception rates declined from 40 to 31%. Month effects were found to have a significant relationship with fertility when climatological measurements were deleted from statistical models. This agreed with most previous research (Stott, 1961; Hafez, 1959). In no case were month effects significant when climatological measurements were included in the model, suggesting that month effects may have represented climatological factors to a greater degree than nutritional and management factors. This work clearly showed the importance of ambient environmental conditions at the time of insemination and fertilization on bovine fertility.

Alterations of peripheral plasma estrogen concentrations during periods of thermal stress have not been reported previously. Therefore, speculation as to estrogenic effects on uterine blood flow and estrogen participation in pre-ovulatory LH release cannot be reviewed here. Physiological and endocrine factors controlling thermal properties of the uterus at estrus and ovulation need clarification. In addition, effects of stressful ambient temperatures on these factors and uterine temperature need further study as they relate to fertility.

Prostaglandins

Prostaglandins (PG), unsaturated 20 carbon fatty acids containing a cyclopentane ring and two alipahtic side chains, were first discovered in extracts of human and sheep seminal vesicles during the 1930's. The first report of their activity before identification was shown when fresh semen was placed into the human uterus and caused the

uterus to contract or relax (Kurzok and Lieb, 1930). The luteolytic effects of $PGF_{2\alpha}$ were reviewed extensively by Inskeep (1973), and effects in cattle well documented by Hafs et al. (1974) and Chenault (1973). Injection of $PGF_{2\alpha}$-Tham Salt in the bovine does not drastically alter the normal sequential hormonal patterns leading to estrus and ovulation. In addition, fertility of cattle to the $PGF_{2\alpha}$ induced ovulation is apparently the same as in a normal spontaneous ovulation of control cattle (Lauderdale et al., 1974). Therefore, $PGF_{2\alpha}$ can be used as an experimental tool to synchronize estrus and investigate physiological and hormonal changes under conditions the researcher wishes to impose. This would be beneficial in large animal research where animal numbers may be few and biological events (the estrous cycle and pregnancy) of long duration. The intention of this portion of the review on prostaglandins is to recapitulate various effects of prostaglandins on the circulatory system and reproductive tract. Such knowledge is essential in evaluating effects of $PGF_{2\alpha}$ in the following experiments.

The role of the autonomic nervous system in controlling uterine contractility and blood flow has been discussed by Shabanah et al. (1964). The parasympathetic system generates uterine contractions and causes vasodilation. The excitatory (α) action of the catecholamines is manifested chiefly on the circular fibers whereas the inhibitory (β) action influences the whole myometrium. Acetylcholine causes vasodilation, especially of smaller blood vessels (Koelle, 1970). Epinephrine is a vasopressor. Vasoconstriction occurs markedly in the venous system, as well as smaller arterioles and precapillary sphincters.

Norepinephrine increases peripheral vascular resistance due to veno-constriction (Innes and Nickerson, 1970). Estrogens govern the parasympathetic (acetylcholine) activity and are responsible for the basic contractile mechanism of the uterus (Shabanah et al., 1964), whereas progesterone influences the sympathetic activity (epinephrine and norepinephrine). Morris (1967) reviewed the sympathetic vaso-constrictor action on the uterine vascular bed. Epinephrine and norepinephrine reportedly cause a decrease in uterine blood flow with a concomitant increase in arterial pressure, suggesting increased vascular resistance in the uterus. Isoproteranol also acts as a vasoconstrictor. These vasoconstrictor actions appeared to be due to increased vascular resistance because myometrial tension changes were negligible.

Clegg (1966) reported that prostaglandins produce two types of effects on smooth muscle. They produce direct short-lived actions such as stimulation of the isolated uterus or relaxation of the isolated tracheal chain preparation. Alternately, they potentiated long-term effects of other stimulants when given in low doses. An example of an indirect long-term effect of prostaglandins (PGF and PGE series) is depression of responses of various isolated smooth muscle preparations to sympathomimetic substances (epinephrine, norepinephrine, phenylephrine and isopropylnoradrenaline).

Different classes of prostaglandins have various effects on smooth muscle and blood pressure and have been reviewed by Bergstrom et al. (1968). PGE's and $PGF_{2\alpha}$ cause contraction of uterine myometrium in rats and guinea pigs. However, in humans, the PGE's decrease tonus, frequency

and amplitude of spontaneous contractions of the myometrium. There is an increase in sensitivity of the rat uterus to $PGF_{2\alpha}$ following estrogen treatment (Anggard and Bergstrom, 1963). $PGF_{2\alpha}$ also has been shown to stimulate and increase tone of the rabbit fallopian tube in vivo (Bergstrom et al., 1968; Horton and Main, 1965), whereas PGE_1 causes relaxation. Isolated strips of human myometrium have a regular motility pattern. This motility in the nonpregnant myometrium was inhibited by PG's A, B and E; however, PG's $F_{1\alpha}$ and $F_{2\alpha}$ stimulated contractions. The sensitivity of the myometrium was highest late in the menstrual cycle and during pregnancy. $PGF_{2\alpha}$ also increased motility of the human oviduct in vivo. Intra-uterine application of $PGF_{2\alpha}$ or intravenous infusion increased the motility of the non-pregnant human uterus (Eliasson, 1973).

Following intramuscular injection of 10 or 20 mg $PGF_{2\alpha}$ in non-pregnant women, no cardiovascular changes were observed but there was pain at the injection site and increased uterine activity within minutes. The uterine contractility lasted 2 to 3 hr. (Karim et al., 1971). Within 1 to 6 hr. after vaginal insertion of PGE_2 or $PGF_{2\alpha}$, 10 to 12 women had menstrual-like uterine bleeding. This bleeding was preceded by a marked increase in uterine contractions which started within 10 min., peaked between 60 to 90 min., and lasted about 4 hr. PGE and $PGF_{2\alpha}$ induced uterine activity that was similar to that recorded for the non-pregnant uterus during the time of the menstrual flow. Contractions measured between 50 to 200 mm Hg and occurred every 1 to 2 min. (Karim, 1971).

In non-pregnant dogs, PGE_1 infused into the uterine artery reduced perfusion pressure. The dilator effect of PGE_1 was seen at doses as

little as 20 pg/ml blood. Such potent vasodilatory effects of PGE_1 were not seen in pregnant dogs near term even with large doses. $PGF_{2\alpha}$, on the other hand, had little effect on vascular smooth muscle in dogs, but potentiated responses to sympathetic nerve stimulation, occasionally in parallel with increased responses to norepinephrine. $PGF_{2\alpha}$ appears to work primarily on nerve terminals in the dog uterus since there was a greater effect on neurogenically induced vasoconstrictor responses than to responses of norepinephrine itself (Clark et al., 1972). In a review by Brody (1973) $PGF_{2\alpha}$ was reported to influence effector response to sympathetic nerve stimulation. Vasoconstrictor action in cutaneous and muscle vessels was facilitated by $PGF_{2\alpha}$ without any change in responsiveness of the vessels to norepinephrine, suggesting that $PGF_{2\alpha}$ facilitated liberation of the adrenergic transmitter. This specificity was not found in venous smooth muscle when $PGF_{2\alpha}$ facilitated responses to both sympathetic nerve stimulation and to norepinephrine. Thus, $PGF_{2\alpha}$ venoconstrictor action was dependent upon integrity of sympathetic innervation.

No changes in cholinergic vasodilator nerves were noted in the presence of prostaglandins (Brody and Kadowitz, 1974). Responses of uterine vessels to norepinephrine were potentiated at $PGF_{2\alpha}$ concentrations which had no effect on uterine vascular resistance.

Recently, Ryan et al. (1974) showed that, in the dog, PGE_1 redistributed the blood flow from the myometrium to the endometrium. Therefore, PGE_1 may be a vasodilator intermediate in an estrogen induced uterine hyperemic response. To test this hypothesis estrogen was injected into rats causing a visible intense hyperemia and a doubling of

uterine blood volume. In comparison, rats pre-treated with indomethacin, a prostaglandin inhibitor, failed to show a large increase in uterine blood volume. In conflict with the observation that $PGF_{2\alpha}$ was a vasoconstrictor was the finding of elevated uterine PGF content following estrogen treatment which could be inhibited by indomethacin pre-treatment. Except for this latter observation, the PGF series appears to be associated with vasoconstrictor actions and reduced blood flow to the uterus.

The cardiovascular actions of $PGF_{2\alpha}$ also are complicated because of quantitative species variation. Anggard and Bergstrom (1963) reported that intravenous injection of $PGF_{2\alpha}$ into cats caused increased right ventricular pressure and a decreased systemic blood pressure. Intra-arterial injections into muscles caused increased blood flow through that area, i.e. vasodilation. $PGF_{2\alpha}$ perfused into rabbit hindquarters also caused tissue vasodilation. Horton and Main (1965) reported that $PGF_{2\alpha}$ or PGE_1 injected intravenously in rabbits caused a fall in arterial blood pressure. A review by Bergstrom et al. (1968) contrasts these results with the pressor action of prostaglandins in the rat, dog and spinal chick. In dogs the pressor action of $PGF_{2\alpha}$ is accompanied by an increase in cardiac output and right atrial pressure, but the calculated peripheral resistance was unchanged. It appeared as if there were a decrease in venous capacitance because when a pressure stabilizer was put into the venous side, it caused a shift of blood into the stabilizer reservoir. Ducharme et al. (1968) reported similar results and also found that, in the dog, $PGF_{2\alpha}$ had little effect on femoral arterial pressure or small artery pressure but caused an increase in small vein

pressure when administered to an innervated limb. Abolishing the sympathetic chain to the limb eliminated the venoconstrictor activity of $PGF_{2\alpha}$. They found no real change in myocardial contractility. Thus the pressor action was due to an increased venous return.

Horton (1969) found PGF to be weakly dilatory on arterioles. In some species (rat and dog) they act as a venoconstrictor, thus increasing venous return and cardiac output. Neither PGE_1 or $PGF_{1\alpha}$ injected close-arterially released catecholamines from the adrenals of anesthetized cats, but PGE_1 did so in dogs. $PGF_{2\alpha}$ injected intra-arterially caused no constant change in blood pressure or in baroreceptor discharge frequency. Moreover, intravenous injections caused a transient rise in arterial pressure which was associated with an increase in baroreceptor discharge. It appeared that the increased discharge frequency was secondary to the pressure rise because in animals where the blood pressure fell slightly, so did discharge frequency. $PGF_{2\alpha}$ injections into the carotid artery resulted in a variable response on chemoreceptor discharges. Intravenously injected $PGF_{2\alpha}$ caused a small increase, decrease or no change at all in blood pressure (McQueen and Belmonte, 1974). Therefore, the authors suggested that direct action was on pressure changes not by way of baroreceptors. These actions may be related to the rapid disappearance of prostaglandins as only 5 to 10% of the injected $PGF_{2\alpha}$ was detected 1 min. later and negligible $PGF_{2\alpha}$ was found at 90 sec. (Raz, 1972). Also, more than 95% of injected prostaglandins were removed during one circulation through the pulmonary vascular bed (Ferreira and Vane, 1967).

Various investigators observed actions of prostaglandins on

respiratory smooth muscle. Main (1964) has shown that PG's E_1, E_2, E_3 and $F_{1\alpha}$ relaxed tracheal muscle in vitro in rabbit, guinea pig (also Puglisi, 1972), ferret, pig, sheep, cat and monkey preparations. Except in the cat, they decreased lung resistance to inflation in vivo (also Anggard and Bergstrom, 1963). $PGF_{2\alpha}$ has similar biological activity to $PGF_{1\alpha}$, so these observations should hold for its actions. This conclusion was confirmed in a cat-trachea preparation by Horton and Main (1965), in which $PGF_{2\alpha}$ inhibited acetylcholine produced contractions. In the dog the action of $PGF_{2\alpha}$ was a reduction in dynamic lung compliance and alveolar ventilation (Horton, 1969).

Investigations on systemic actions of prostaglandin in the bovine are very limited to date. Lewis and Eyre (1972) reported that PGE_1 and E_2 lowered systemic blood pressure in calves, but $PGF_{2\alpha}$ caused a pressor response. Furthermore, pulmonary arterial pressure and abdominal venous pressure were raised by the three substances. $PGF_{2\alpha}$ caused contraction of the pulmonary artery and vein and produced an increase in heart rate. Also noted was an increase in respiratory volume produced by $PGF_{2\alpha}$. Anderson et al. (1972) also concurred that $PGF_{2\alpha}$ increased pulmonary arterial pressure, but they found a drop in cardiac output and essentially no change in femoral arterial pressure, left ventricle and diastolic pressure, heart rate, blood gases and pH.

There is a definite need for more study on actions of prostaglandins to determine their roles in physiological functions in the bovine. Additional work is needed because of the contradictory results obtained among and within species.

Hormone Relationships of Uterine Blood Flow and Temperature

The uterus responds to cyclic hormonal changes during the estrous or menstrual cycles. Blood levels of estrogen and progesterone are involved with this phenomena. In the human, the first half of the cycle is associated with rapid growth of the uterine vascular elements and is under estrogenic control. This is a period of tissue repair and proliferation. The latter half of the cycle is characterized by glandular secretory activity and elaboration of vascular elements under the control of estrogen and progesterone (Reynolds, 1949).

One of the principal characteristics of the uterus following estrogen administration is its bright red color. The degree of redness suggests a high level of oxygen saturation of the blood and there is a high rate of blood flow. In the presence of an active corpus luteum (progestational influence), the uterus is bluish in color. Oxygen consumption is low and blood flow is sluggish. Oxytocin causes intense muscular spasms within the uterus without affecting the rate of blood flow, whereas vasopressin causes relaxation of uterine musculature but a constriction of its vasculature (Reynolds, 1949).

Uterine hyperemia following injection of estrogen has been estimated in ewes by direct collection of uterine venous blood (Huckabee et al., 1970), by flow meters (Greiss and Anderson, 1970; Rosenfeld et al., 1973) and microspheres (Rosenfeld et al., 1973). Endogenous estrogens produced during the estrous cycle appear to have similar effects on uterine blood flow. Patterns of change in plasma estradiol concentrations (Scaramuzzi, Caldwell and Moor, 1970) are very similar to records of

uterine blood flow changes in the ewe (Greiss and Anderson, 1970; Huckabee et al., 1968, 1970). Specificity of estrogen actions on uterine blood flow have been shown by local injection of estrogen into one uterine horn artery. An increase in blood flow was measured only in that uterine artery (Resnik et al., 1974).

Progesterone injected into ovariectomized ewes did not alter uterine blood flow, whereas progesterone superimposed on estradiol injections caused a decrease in uterine blood flow rates (Greiss and Anderson, 1970). Estrogens did not appear to affect systemic blood pressure (Huckabee et al., 1968, 1970; Resnik et al., 1974), but caused a fall in the coefficient of oxygen utilization $[\frac{(A-V)O_2}{AO_2} \times 100]$ in the uterus. However, due to the higher uterine blood flow there was essentially no change in oxygen uptake of the uterus (Huckabee et al., 1968, 1970). Thus a dissociation between uterine metabolic rate and the rate of blood flow might be reflected in temperature differences between the uterus and aortic blood. In sheep a decrease in the temperature difference between the uterine cavity and aortic blood provided a convenient method for monitoring increased uterine blood flow changes following estrogen injection. A rise in blood flow resulted in a lowered uterine temperature (Abrams et al., 1970a, 1971). The actions of estrogen to lower uterine temperature may be mediated through its interaction with acetylcholine to cause vasodilation (Shabanah et al., 1964) or through the release of uterine histamine which was shown to be involved in a rapid onset of hyperemia and water imbibition (Jensen and DeSombre, 1972). Lowering the rate of uterine heat production is unlikely because of the many metabolic activities induced by estrogens

(Talwar and Segal, 1971; Jensen and DeSombre, 1972).

In cattle, plasma estrogens increasedprior to estrus and declined precipitously during estrus (Chenault et al., 1973; Henricks, Dickey and Hill, 1971). These changes may have distinct thermal effects on the uterus. Greiss and Anderson (1969) reported increased uterine blood flow associated with the onset of estrus in sheep, which could cause a drop in uterine temperature (Abrams et al., 1970a, 1971; Caton et al., 1974) and thus be related to an optimal time for insemination to achieve maximal fertility. However, the thermal response of the bovine uterus to estrogen has not been documented.

Although several hormonal changes due to hyperthermia have been documented in the bovine there is a sparcity of results related to a multiplicity of hormonal responses to a controlled thermal stress in which such sources of variation due to breed, age, animal and time responses are evaluated. Uterine blood flow and temperature relationships have been reported in sheep in response to estrogen injections, but have not been reported in the bovine. Also, changes in uterine temperature during phases of the estrous cycle under conditions of mild heat stress have not been reported.

SECTION II

HORMONAL PATTERNS DURING HEAT STRESS FROM $PGF_{2\alpha}$ INJECTION THROUGH ESTRUS AND OVULATION AND FOLLOWING ADRENAL STIMULATION BY ACTH IN HEIFERS

Introduction

Lowered conception rate due to heat stress occurs over a prolonged period of the year in tropical and subtropical climates. Before systems for reproductive management can be developed to counter these adverse effects of heat stress, a more complete understanding of endocrine and physiological changes within the same animals must be made. We need to know how a standard heat stress alters hormonal and physiological responses during the normal estrous cycle, and determine if chronic heat stress alters adrenal responsiveness to an IV injection of ACTH.

Due to limitations of facilities and methodology, most investigations of heat stress effects on hormonal balance and their relationships to reproductive performance have monitored only one or two hormonal responses. Therefore, pooling results from different laboratories and from different animals within and between species can be misleading when an effort is made to develop a hypothesis on how thermal stress affects reproduction.

Reduced fertility in hot environments was associated with elevated body temperature (Dunlap and Vincent, 1971; Gwazdauskas, Thatcher and Wilcox, 1973). Hot climates may exert their depressive effects on fertility by acting on the gonads, uterine environment, endocrine system or gametes (Hafez, 1959; Ulberg and Burfening, 1967). Seasonal infertility has been attributed primarily to the bovine female (Stott, 1961). Studies on hormonal alterations due to thermal stress have failed to document interrelationships of more than two different hormones in the same animals. Plasma progestin changes have been documented by Mills et al. (1972), Abilay and Johnson (1973) and Abilay, Johnson and Seif (1973); changes in plasma corticoid levels have been reported by Lee, Roussel and Beatty (1973), Christison and Johnson (1972), Abilay and Johnson (1973), Shayanfar (1973) and Miller and Alliston (1974a). Plasma LH changes have been reported by Madan and Johnson (1971) and Miller and Alliston (1973) and seasonal changes in prolactin have been detected by Koprowski and Tucker (1973), Schams and Reinhart (1974) and Thatcher (1974). Such hormonal alterations may be causative agents contributing to suppressed estrous manifestation and depressed fertility under hot climatic conditions.

There are essentially no studies designed to test specific effects of heat stress environments on a multiplicity of hormonal responses within the same animal. Such a study is needed in which variations due to breed, age, animal (among and within) and hormonal interrelationships are considered in evaluating thermal stress effects.

Objectives of this study were to characterize changes in peripheral plasma concentrations of LH, progestins, estradiol, estrone, prolactin

and corticoids after an intramuscular (IM) injection of $PGF_{2\alpha}$-Tham Salt ($PGF_{2\alpha}$) under controlled environmental temperatures (21.3 C compared to 32.0 C), and to determine if chronic heat stress alters adrenal responsiveness to an intravenous (IV) injection of ACTH (200 IU).

Materials and Methods

Ten normally cycling Holstein heifers at the USDA, Agricultural Research Center, Beltsville, Maryland, were assigned alternately, based on age, to one of two treatment groups (figure 1). All heifers were placed in one of two environmental chambers at 21.3 C, 59% RH for 2 weeks. On day 9 of this adaptation period, 8 of 10 heifers in the luteal phase of the estrous cycle received 30 mg $PGF_{2\alpha}$[a] (IM) to cause luteal regression. This injection allowed all heifers to be in the luteal phase of the cycle when $PGF_{2\alpha}$ was injected 12 days later. $PGF_{2\alpha}$ effectively regresses the bovine corpus luteum and synchronizes estrus. Lauderdale et al. (1973, 1974), Louis et al. (1974) and Chenault et al. (1974) reported that fertility at the synchronized estrus, and induced hormonal changes resulting in estrus and ovulation appeared normal in $PGF_{2\alpha}$ treated cattle. Thus, it was felt that $PGF_{2\alpha}$ treatment could be utilized as a tool to best control reproductive status of the heifers and maximize efficient use of the chambers.

On day 14, the environment of one chamber was adjusted to 32.0 C, 67.2% RH. On day 20, all heifers were fitted with indwelling polyvinyl jugular catheters (V-7; Bolab Inc., Derry, N.H.) and $PGF_{2\alpha}$ (30 mg, IM)

[a] $PGF_{2\alpha}$-Tham Salt was graciously supplied by Dr. J. W. Lauderdale, Upjohn Co., Kalamazoo, Michigan.

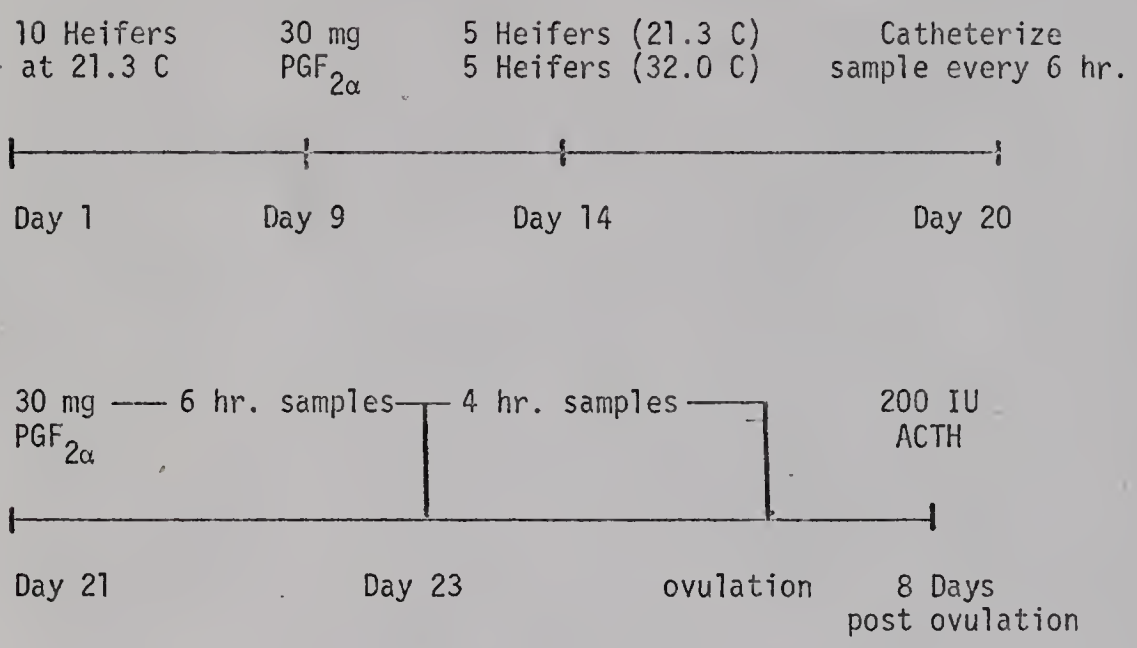

Figure 1. Evaluation of thermal stress on transitory hormonal changes in the bovine during the period of luteal regression, estrus and ovulation: Experimental design.

was given on day 21. Such treatment would allow for monitoring of hormonal responses associated with corpus luteum regression, follicle growth and ovulation under two different environments (21.3 C compared to 32.0 C).

Blood samples (50 ml) were collected from jugular catheters at -18, -12 and 0 hr. pre-$PGF_{2\alpha}$ (day 21), at 6 hr. intervals for 48 hr. post-injection, every 4 hr. thereafter until ovulation, and then twice daily until the last heifer ovulated (figure 1). All blood was collected into heparinized syringes, placed immediately into an ice bath, centrifuged at 12,000 g for 10 min. at 4 C, and plasma stored at -20 C until analyzed for progestins, LH, estradiol, estrone, corticoids, prolactin, protein concentration, osmolarity and cortisol binding capacity.

Beginning 48 hr. post-$PGF_{2\alpha}$ injection, animals were checked visually for estrus at 4 hr. intervals. Heifers were artifically inseminated 12 hr. after onset of estrus, and ovulation determined by rectal palpation of an ovarian ovulatory crypt. Palpations were performed at 4 hr. intervals following cessation of estrus.

Chamber temperatures and relative humidities were recorded continuously (Honeywell Recorder, Washington, Pa.), and temperature of each chamber verified daily with a tele-thermometer [Model 46 TUC, Yellow Springs Instrument Co., Inc. (YSI), Yellow Springs, Ohio; air temperature probe T 2620 (YSI)]. Rectal temperatures were monitored daily (tele-thermometer probe T 2600, YSI). Skin temperatures taken on the shoulder, rump and approximately 5 to 8 cm lateral to the vulva (surface temperature probe T 2630, YSI) also were monitored daily during the serial blood collection period. Thermister probes were calibrated

against a Bureau of Standards Certified Thermometer in a well-stirred, insulated water bath held at 35 to 40 C. Data collected from the various probes were corrected for constant temperature differences above and below the certified thermometer readings.

Plasma samples were pooled within heifers (after the drop of the preovulatory LH peak to basal levels and having less than 3 pg/ml estradiol) to determine total protein concentration (Lowry method), osmolality, (freezing point depression, Fiske Osmometer, Model G-61, Fiske Ass., Inc., Bethel, Conn.), cortisol binding capacity and cortisol association constants (Pegg and Keane, 1969; Shayanfar, 1973) for each heifer.

In the second phase of the trial adrenal responsiveness to ACTH was tested. Eight days after the last heifer ovulated, all heifers received 200 IU ACTH (Porcine ACTH, Sigma Chemical Co., St. Louis, Mo.). Blood samples (50 ml) were collected from indwelling jugular catheters at -2, -1, 0 hr. pre-injection, 15, 30, 45, 60 min. and hourly thereafter up to 12 hr. postinjection.

LH and prolactin were assayed in plasma at two dilutions using the double antibody radioimmunoassay (RIA) of Niswender et al. (1969). Guinea pig antibovine LH serum (Oxender, Hafs and Edgerton, 1972) was supplied by Dr. H. D. Hafs of Michigan State University and revalidated with NIH-LH-B7 for measuring plasma LH in our laboratory (Troconiz, 1973). Guinea pig anti-bovine prolactin serum (Koprowski and Tucker, 1971) was donated by Dr. H. A. Tucker of Michigan State University and revalidated for measuring plasma prolactin with NIH-P-B3 prolactin (Chakriyarat, 1974 personal communication). Plasma progestins,

estradiol and estrone were measured by RIA procedures described by Abraham et al. (1971) and Hotchkiss et al. (1971), respectively. The antiprogesterone antibody was a gift from Dr. K. Kirton of the Upjohn Co., and the estrogen antibody was donated by Dr. V. L. Estergreen of Washington State University. Extraction, purification and quantitative procedures were validated in our laboratory by Chenault et al. (1973, 1974, 1975). Plasma corticoids were extracted, isolated and quantified by competitive protein binding (Gwazdauskas, Thatcher and Wilcox, 1972, 1973). The only modification was use of a .2 ml dextran coated charcoal suspension (100 mg Dextran, Type 60 C, Sigma Chemical Co., St. Louis, Mo.; 1 gm Norit A, Sigma Chemical Co. and 100 ml deionized water) instead of 80 mg florisil for adsorption of free steroid in the competitive protein binding assay.

An extensive series of least-squares analyses was conducted to characterize treatment, animal and within-animal time trends in plasma LH, progestins, estradiol, estrone, prolactin and corticoids. Other response variables were analyzed by analysis of variance.

Results and Discussion

Averages and standard deviations for chamber conditions, rectal and skin temperatures, and events associated with estrus are shown in table 1. Based on Christison and Johnson's (1972) criteria for a moderate heat stress condition (rectal temperature increase of .5 C) climatic conditions of our study exerted a greater than moderate heat stress since rectal temperatures of heifers in the 32.0 C chamber were

Table 1. Physiological parameters of heifers in environmental chambers at 21.3 C and 32.0 C.

Air temperature	21.3[a] ± .75[c] C	32.0 ± .48[d] C
Relative humidity	58.9 ± 3.3[c] %	67.2 ± 3.5[d] %
Rectal temperature	38.75 ± .23[c] C	40.24 ± .33[d] C**
Shoulder temperature	32.60 ± .97[c] C	36.54 ± .47[d] C**
Vulval temperature	33.68 ± .71[c] C	36.83 ± .68[d] C**
Rump temperature	33.29 ± .89[c] C	36.97 ± .45[d] C**
$PGF_{2\alpha}$ to LH peak	94.4 ± 26.2[e] hr.	72.8 ± 23.2[e] hr.
$PGF_{2\alpha}$ to ovulation	118.4 ± 23.9[e] hr.	96.0 ± 24.8[e] hr.
Estrus length	21.0 ± 3.8[f] hr.	16.0 ± 3.7[e] hr.[b]

[a] ($\bar{X}$ ± SD)
**($P<.01$)
[b] ($P<.10$)
[c] ($n=26$), [d] ($n=23$), [e] ($n=5$), [f] ($n=4$)

1.49 C greater than heifers in the 21.3 C chamber. Skin temperatures also were significantly ($P<.01$) elevated in the hot chamber. Visual appraisal of the data showed there was no tendency for skin or rectal temperatures to decline during chronic exposure to the heat stress of our experiment which would have suggested adaptation. Indices of physiological response to $PGF_{2\alpha}$ (duration of time between $PGF_{2\alpha}$ injection and the LH peak and time between $PGF_{2\alpha}$ injection and ovulation) were not different between treatments ($P>.10$). The interval from the LH peak to ovulation was approximately 24 hr. for both groups (24 hr. in 21.3 C; 23.2 hr. in 32.0 C). This interval is similar to that reported by Chenault et al. (1973, 1975). Arije, Wiltbank and Hopwood (1974), and Christenson, Echternkamp and Laster (1974) for unsynchronized animals and the $PGF_{2\alpha}$ induced interval reported by Chenault et al. (1974) and Hafs et al. (1974). If the trend for the heat stress group to have shorter intervals from $PGF_{2\alpha}$ injection to LH peak and ovulation is real (table 1), we may have failed to detect differences due to small numbers ($n=5$ each) and appreciable variation. In this study, if hyperthermia affected endocrine-physiological interactions, it did not appear to alter time between the LH peak and ovulation.

Length of estrus was significantly shorter ($P<.10$) for the heat stressed heifers and was comparable to the 14 hr. duration of estrus reported by Gangwar, Branton and Evans (1965) for Holstein heifers in hot natural summer climatic conditions of Louisiana. Two of four heifers inseminated in the 21.3 C chamber were pregnant at 40 days compared to none of 5 in the 32.0 C chamber. Though there were small numbers of animals inseminated in this trial, the percentage of successful pregnancies

was comparable to that of the Dunlap and Vincent (1971) environmental chamber study. This related well to observations that thermal stress in this study interfered with the overall reproductive process in heifers. Thus, the environmental condition did affect body temperature, duration of estrus and probably overall fertility. Whether hormonal response under these conditions varied was of utmost interest.

Pre-$PGF_{2\alpha}$ injection plasma samples were analyzed by analysis of variance to detect possible differences in progestins, estradiol, estrone, LH, prolactin and corticoids due to temperature, sampling time, temperature X sampling time interaction and animals within temperature treatment. Progestins ($\overline{X}$ = 3.21 ng/ml, 21.3 C; $\overline{X}$ = 3.16 ng/ml, 32.0 C) were not influenced by temperature or sampling time. However, a significant ($P<.05$) temperature by time interaction suggested different progestin concentrations at different times of sampling in each treatment chamber. Plasma progestins appeared to decline in the heat stressed group with progressive sampling, whereas in the cool group they did not change (Appendix, table 12). Also, there was significant ($P<.01$) variability in progestin concentrations among heifers in each chamber. This would suggest that there is considerable variation in progestin secretion from heifers during the luteal phase of the cycle. Animal variability in pre-injection plasma estradiol concentrations ($\overline{X}$ = 3.02 pg/ml, 21.3 C; 2.32 pg/ml, 32.0 C) was significant ($P<.01$). Estrone ($\overline{X}$ = 3.04 pg/ml, 21.3 C; $\overline{X}$ = 3.25 pg/ml, 32.0 C), LH ($\overline{X}$ = .53 ng/ml, 21.3 C; $\overline{X}$ = 182 ng/ml, 32.0 C) and prolactin ($\overline{X}$ = 12.73 ng/ml, 21.3 C; $\overline{X}$ = 15.32 ng/ml, 32.0 C) pre-$PGF_{2\alpha}$ plasma concentrations were not influenced by temperature, time of sampling, temperature X time interaction or animals within temperature

treatment (P>.10). Variability in plasma corticoids was significant due to sampling time (P<.01) and treatment X sample time interaction (P<.01). However, there was no difference due to main effects of temperature (9.0 ng/ml, 21.3 C; 9.55 ng/ml, 32.0 C).

A logical physiological reference to analyze the data is the preovulatory surge of LH. All animals had a preovulatory LH surge, and each hormone was analysed initially to determine time relationships with the LH surge. Least squares statistical models were selected based on tests of significance of higher order terms (time) in the regression analyses and visual appraisal of the graphs.

Figure 2 shows the progestin responses at 21.3 C and 32.0 C. Data for the regression analyses have been synchronized to the LH peak for analysis. The statistical model included treatment, heifer within treatment and time trends (Appendix, table 4). The progestin time trend for heifers at 21.3 C was best described by the equation $\hat{Y}$ (progestin, ng/ml) = $1.533 + 1.160X - .344X^2 + .034X^3 - .0014X^4 + .00002X^5$ (P<.01) where X = .1 hr., whereas the time trend for the 32.0 C heifers showed a significant (P<.01) curvilinear relationship best described by a third order equation: $\hat{Y} = 7.923 - 1.123X + .061X^2 - .0010X^3$.

Tests for heterogeneity of regression were significant (P<.01), suggesting that the 5th order regression curves for each treatment were not parallel. This observation implies that there was a different time response to treatments. We feel that this difference was probably due to heifers in the 21.3 C chamber having their LH peak approximately 22 hr. later from the start of blood sampling than the heifers maintained at 32.0 C (table 1). Therefore, on the average cool heifers had

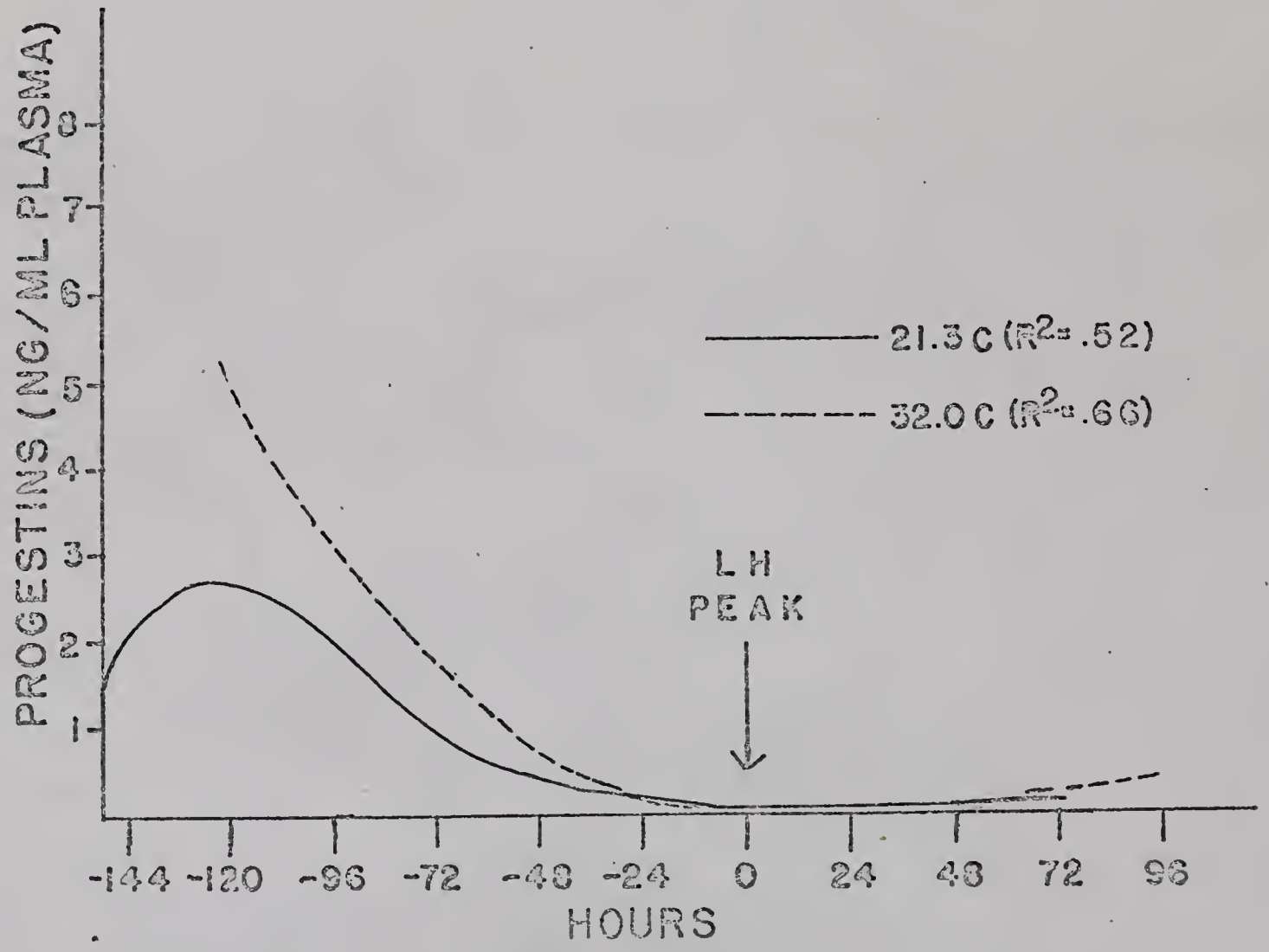

FIGURE 2. SEQUENTIAL CHANGES IN PLASMA PROGESTINS IN HEIFERS AT 21.3 C OR 32.0 C SYNCHRONIZED TO THE TIME OF THE LH PEAK.

a longer plateau of low progestins prior to the LH peak. $PGF_{2\alpha}$ caused a drop in progestin concentration by 18 hr. after injection in all heifers (Appendix, table 5). Apparently, factors controlling the pre-ovulatory release of LH in cool heifers were slightly delayed (~ 24 hr.) due either to treatment effects or chance. Thus, due to a shorter interval between $PGF_{2\alpha}$ and the LH peak for the 32.0 C heifers, one would expect higher progestins because of shorter trough duration. Also, the number of observations earlier than -120 hr. was very small. Significant ($P<.01$) among heifer differences were detected within treatments, but differences among treatments were not significant [($P>.10$) Appendix, table 4). This finding is in direct conflict with Stott, Thomas and Glen (1967), who reported elevated progesterone on day of estrus in thermally stressed cows. However, Stott and Wiersma (1973) more recently reported a depression in plasma progestins due to chronic thermal stress.

The progestin RIA has a sensitivity of 25 pg or .025 ng/ml plasma. Coefficients of Variation (C.V.) for progestins after accounting for variability due to treatment, heifer in treatment and time trends were 115% (cool) and 99% (hot). Acute increases of 2.5 (Gwazdauskas, Thatcher and Wilcox, 1972) and 1.5 ng progesterone per ml plasma (Wagner, Strohbehn and Harris, 1972) for a period of 2 hr. were detected following 200 IU and 100 IU ACTH injections, respectively. If thermal stress had elicited an adrenal release of progesterone we would have been able to detect it. Mills et al. (1972) detected a significant elevation of only .47 ng/ml progestins in heifers thermally stressed for 72 hr. at the onset of estrus. Therefore, with overall progestin levels of

.147 $\pm$ .095 ng/ml ($\bar{X} \pm SD$) the day of estrus and day after estrus and no differences due to temperature, we cannot conclude that chronic heat stress caused adrenal hyperprogesterone response. Our findings support Miller and Alliston (1974b), who found no difference in plasma progesterone during the bovine estrous cycle when twice daily measurements were made in control (17-21 C) or heat stress (21-34 C) environments. Progestins do not appear to be elevated during the period between luteal regression and ovulation.

Data for regression analyses of estradiol were separated into two periods and independently analyzed to characterize time trends. The two periods were from -144 hr. to time of the LH peak (including LH peak time) and from the LH peak to +96 hr. (also including the LH peak time). Pre and post LH peak time trends for estradiol were best described by $\hat{Y}_{pre}$ (estradiol, pg/ml) = $4.53 - .482X - .045X^2 + .0069X^3$ ($P<.05$) and $\hat{Y}_{post} = 45860.38 - 12165.711X + 1285.829X^2 - 67.674X^3 + 1.773X^4 - .019X^5$ ($P<.05$) for the cool heifers and $\hat{Y}_{pre} = 5.67 - 1.279X + .092X^2$ ($P<.01$) and $\hat{Y}_{post} = 490.168 - 73.503X + 3.647X^2 - .060X^3$ ($P<.01$) for the hot heifers (figure 3). Plasma estradiol was depressed ($P<.10$) in the 32.0 C chamber (peak estradiol:10.4 pg/ml plasma for 21.3 C heifers compared to 7.2 pg/ml plasma for 32.0 C heifers; Appendix, tables 4 and 6).

Lower plasma estradiol may have contributed to the shorter periods of estrus seen in the 32.0 C heifers. However, these lower concentrations of estradiol were adequate to elicit estrous behavior and LH release causing a subsequent ovulation. The lower estradiol may reflect altered production, secretion, clearance or receptor binding under

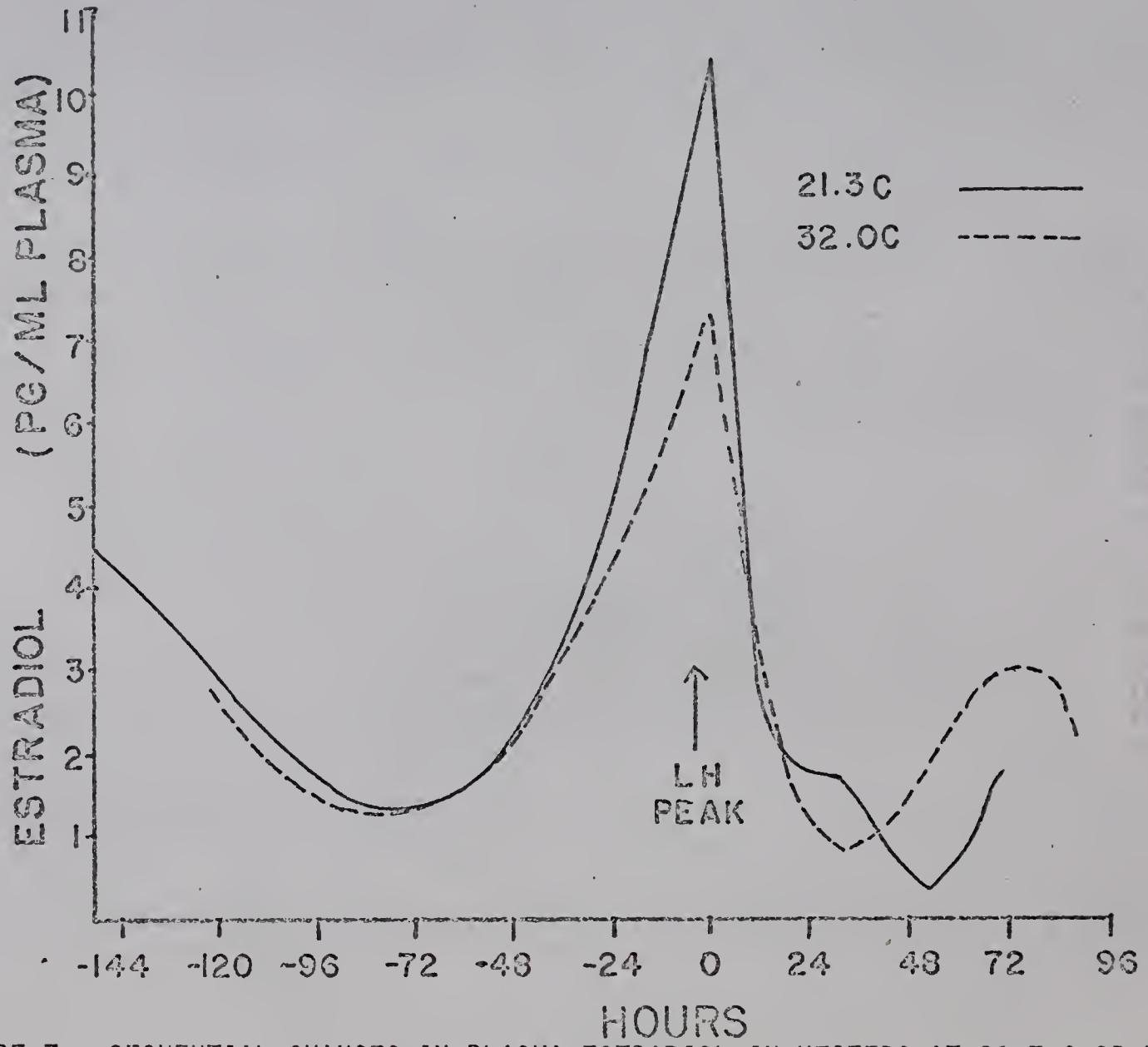

FIGURE 3. SEQUENTIAL CHANGES IN PLASMA ESTRADIOL IN HEIFERS AT 21.3 C OR 32.0 C SYNCHRONIZED TO THE TIME OF THE LH PEAK.

conditions of hyperthermia in cattle. Significant among heifer variability ($P<.01$) was detected in the 32.0 C heifers (Appendix, table 4). Whether slightly altered plasma estradiol would affect such factors as uterine and oviductal blood flow, temperature of the reproductive tract, tract motility, gamete and embryo transport that may contribute to poor fertility under heat stress is not known. Results of the present study reveal only a subtle effect of heat stress on plasma estradiol.

Unlike the time responses of estradiol, estrone showed no apparent association with onset of estrus or LH peak when data were synchronized to the time of the LH peak ($r\sim0$, estrone:estradiol; table 2). Hansel (1971) reported an estrone peak between days 13 to 16 of the bovine estrous cycle and suggested that this elevation in estrone may be related to corpus luteum regression. Therefore, data were analyzed from the time of the $PGF_{2\alpha}$ injection (figure 4; Appendix, tables 4 and 7). Time responses were best characterized by $\hat{Y}$ (estrone, pg/ml) = $2.671 + 1.488X - .763X^2 + .120X^3 - .0077X^4 + .00018X^5$ ($P<.01$) for the 21.3 C group and $\hat{Y} = 2.298 + 1.828X - .781X^2 + .145X^3 - .0072X^4 + .00017X^5$ ($P<.05$) for the 32.0 C heifers. The significant elevation ($P<.05$) of estrone (Appendix, tables 4 and 7) due to heat stress is best seen following $PGF_{2\alpha}$ through 72 hr. (figure 4). There was no evidence that these curves were not parallel (5th order, $P>.10$) suggesting that in both treatments estrone followed the same decline post-$PGF_{2\alpha}$. However, estrone levels were higher through 72 hr. in heat stressed heifers. The slight rise in estrone prior to $PGF_{2\alpha}$ injection to +12 hr. may be related to a luteolytic action as suggested by Hansel (1971), although heifers were only day 9 of the estrous cycle at time of $PGF_{2\alpha}$ injection.

Table 2. Simple correlations between hormone measurements[a].

	Progestins	Estradiol	Estrone	Corticoids	Prolactin
LH	-.14*	.45**	.03	-.02	-.01
Progestins		-.14*	.40**	.14*	-.07
Estradiol			.00	.11*	.02
Estrone				.09	-.06
Corticoids					.22**

[a] n=291
*(P<.05), >.11
**(P<.01), >.15

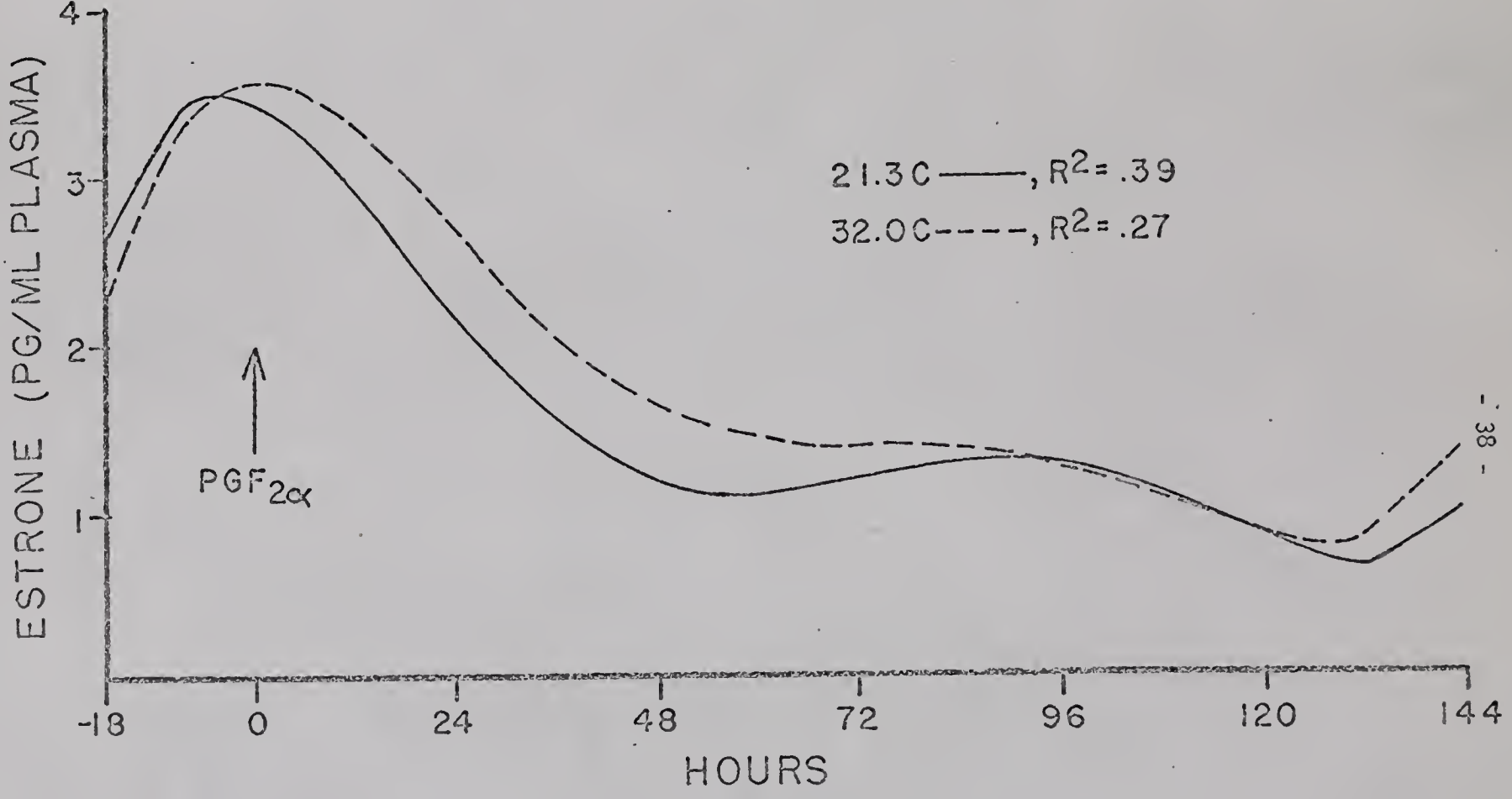

FIGURE 4. · SEQUENTIAL CHANGES IN PLASMA ESTRONE IN HEIFERS AT 21.3 C OR 32.0 C SYNCHRONIZED TO THE TIME OF $PGF_{2\alpha}$ INJECTION.

Chenault et al. (1974) and Henricks et al. (1974) reported estrone to vary greatly within and among animals after $PGF_{2\alpha}$ injections. The C.V.'s for estrone in this study after accounting for heifer and time variability were 65.3 and 74.0% for cool and hot groups, respectively. Plasma estrone concentrations were less than 5 pg/ml and agree with the work of Echternkamp and Hansel (1973). However, they reported that estrone was slightly elevated at estrus in one cow. This observation was difficult to support since there was no statistical analysis or report of estrone variability.

Because LH increases above basal levels for only about 10 hr. (Chenault et al., 1975), LH data were separated into four time periods to analyze, independently, both basal and preovulatory peak concentrations. These periods were: (1) from -148 to 8 hr. prior to the LH peak (-8 hr.); (2) -8 hr. to the peak of LH; (3) LH peak to +8 hr. and (4) +8 hr. to +96 hr. (figure 5). Average LH concentrations for period (1) were 1.80 $\pm$ 1.21 ng/ml plasma ($\bar{X} \pm$ SD; n=102) for the cool heifers (21.1 C) compared to 1.75 $\pm$ 1.27 ng/ml (n=74) for the heat stressed heifers (32.0 C). A linear increase ($P<.01$) in LH occurred between -8 hr. to the LH peak (peak LH 32.19 ng/ml for cool heifers compared to 33.17 ng/ml for hot heifers), and then dropped linearly ($P<.01$) to basal levels by +8 hr. (.43 ng/ml - cool compared to .49 ng/ml - hot). Basal levels were defined as any LH concentration within three standard deviations of the mean for all samples (n=169) up to -8 hr. (1.75 $\pm$ 1.22 ng/ml). During the initial period, two heifers in the cool chamber had sporadic peaks (>3 S.D.) of LH that occurred between -72 and -12 hr. prior to the LH peak (Appendix, table 8).

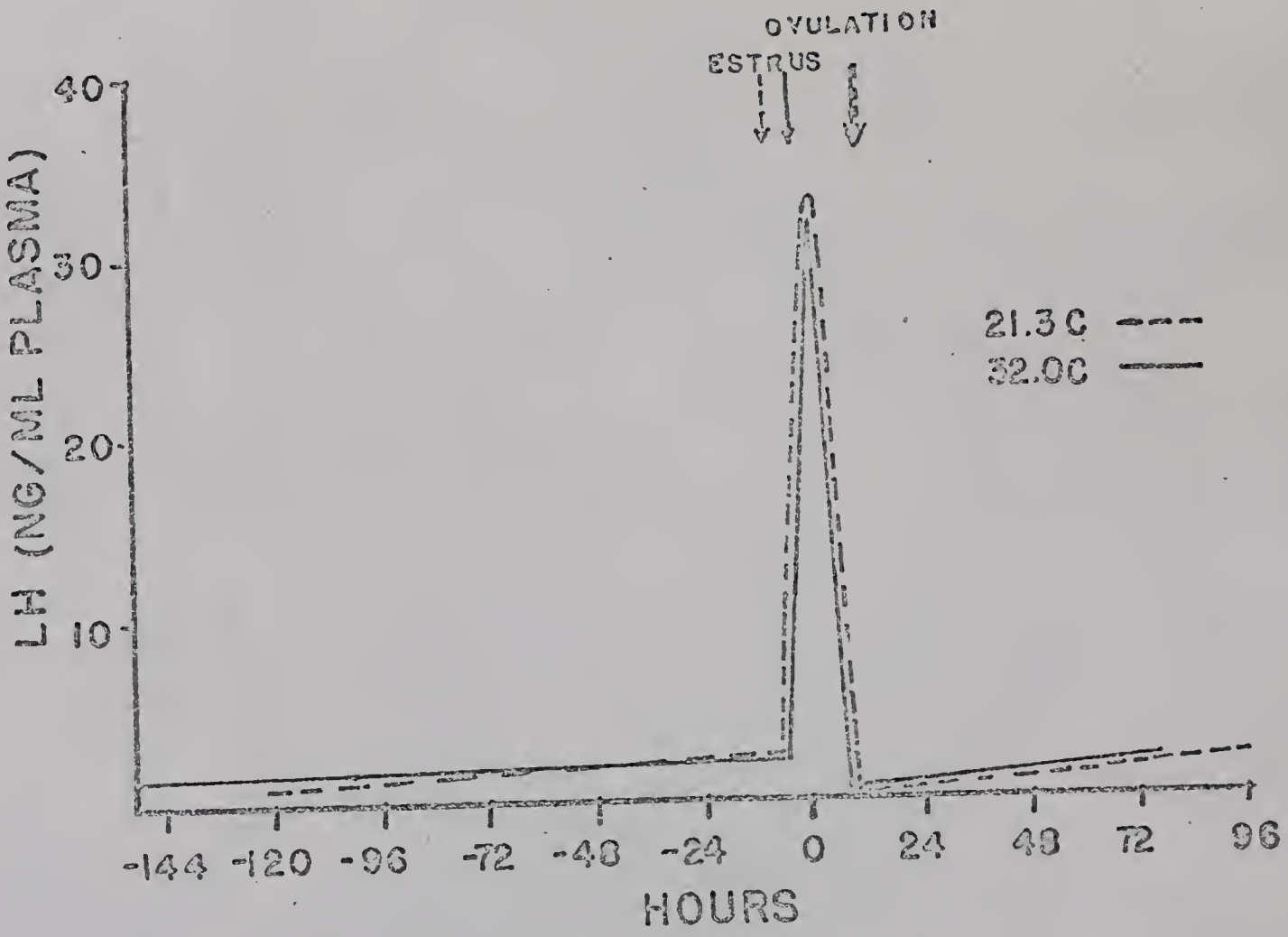

FIGURE 5. SEQUENTIAL CHANGES IN PLASMA LH IN HEIFERS AT EITHER 21.3 C OR 32.0 C.

The preovulatory surge of LH remained above basal levels for 10.4 and 9.6 hr. for the 21.3 C and 32.0 C groups, respectively. LH concentrations in this study agree with those of Henricks, Dickey and Niswender (1970) and Snook, Saatman and Hansel (1971). Unlike results of Madan and Johnson (1971) and Miller and Alliston (1973), we found no significant difference in LH levels in response to heat stress (Appendix, table 4). Our contradictory results under conditions of thermal stress may be due to frequency of sampling (twice daily; Miller and Alliston, 1973), sensitivity of experimental design in which among animal variability was considered in the present study, duration of the LH peak (~ 10 hr.) or breed differences (Madan and Johnson, 1971). Riggs, Alliston and Wilson (1974) detected a difference in the preovulatory LH surge during heat stress between Hampshire and Duroc gilts. Such differences may exist between the study of Madan and Johnson (1971) in which Guernsey cattle were used and in our study where only Holstein heifers were used.

All animals had a preovulatory LH surge, suggesting that hyperthermia did not prevent the triggering mechanism for LH release. Although estradiol levels in peripheral plasma were slightly depressed in the 32.0 C heifers, it does not appear that these lowered estradiol concentrations altered LH release.

Plasma prolactin was analyzed initially in the same manner as estradiol (pre- and post peak of LH). However, there was no change in prolactin associated with estrus or the LH peak as previously reported by Swanson and Hafs (1971). Absence of any association between prolactin and estrus is supported by work of Hoffman et al. (1974) in which an

inhibitor of prolactin secretion caused no estrous cycle disorders. Also, Wetteman and Hafs (1973) were unable to find elevated prolactin on the day of estrus.

Since there were no detectable changes in prolactin associated with the LH peak, data were analyzed further relative to time of $PGF_{2\alpha}$ injections. Hafs et al. (1974) reported that prolactin increased immediately following $PGF_{2\alpha}$ injection (within 1 hr. and lasting for 4 hr.). However, an increase in our study was not detected because the first blood samples were not taken until 6 hr. after injection. No differences were detected between the 21.3 C and 32.0 C treatment groups ($P>.10$; Appendix, tables 4 and 9). Time trends of prolactin for the 21.3 C and 32.0 C treatment groups were described by the following equations: $\hat{Y}$ (prolactin, ng/ml) = $14.50 - 1.707X + .371X^2 - .018X^3$ and $\hat{Y} = 14.28 + 2.738X - .733X^2 + .066X^3 - .002X^4$ (32.0 C) (figure 6). The 4th order time curves were not parallel ($P<.005$). Prolactin C.V. after accounting for heifers and the above time equations was 49.4% (21.3 C) and 26.3% (32.0 C). During the initial 42 hr. (-18 to 24 hr.), the prolactin response in the cool chamber appeared to decline. This observation may be due to a lowering of stress-induced prolactin secretion with more sampling (Tucker, 1971). Apparently heifers in the 32.0 C chamber could not adjust to sampling as quickly since prolactin increased and remained elevated until 24 hr. after $PGF_{2\alpha}$. However, this is questionable since trends are very subtle and the curves account for little of the variability (figure 6).

An increase in plasma prolactin due to heat stress was anticipated in the present study based on reports of Koprowski and Tucker (1973),

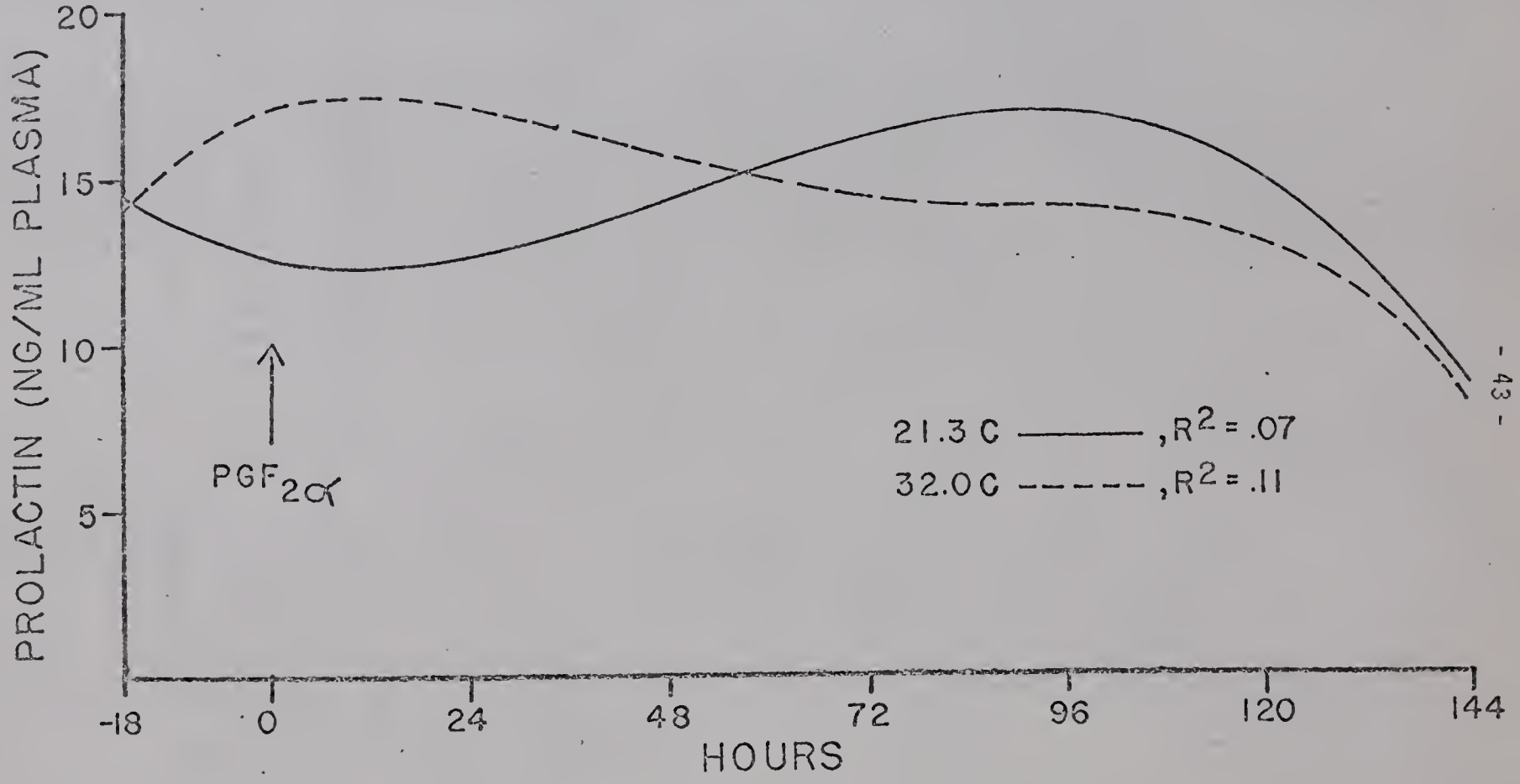

FIGURE 6. SEQUENTIAL CHANGES IN PLASMA PROLACTIN IN HEIFERS AT 21.3 C OR 32.0 C SYNCHRONIZED TO THE TIME OF $PGF_{2\alpha}$ INJECTION.

Schams and Reinhart (1974) and Thatcher (1974), in which seasonal changes of plasma prolactin were detected (high during summer). Wetteman and Tucker (1974), using twice daily sampling, detected only slight differences ($P<.10$) in serum prolactin in 3 mo. old calves exposed either to 21 or 27 C temperatures under a 12 hr. per day light regime. The induced release of prolactin following injection of thyrotropin releasing hormone (TRH) was twice as great in calves exposed to 27 C as calves at 10 C. Furthermore, they suggested that these results, which are opposite those seen in lactating cows following the milking stimulus, may be due to differences in anterior pituitary responsiveness of 3 mo. old calves at different temperatures.

In our study, no differences in average plasma prolactin concentrations were detected between heifers at 21.3 C or 32.0 C (figure 6; Appendix, tables 4 and 9). Thus at a constant 14 hr. light - 10 hr. dark regime an environmental temperature of 32.0 C caused no increase in prolactin compared to controls at 21.3 C. Perhaps other factors control the seasonal increase in prolactin previously reported (Koprowski and Tucker, 1973; Schams and Reinhart, 1974; Thatcher, 1974). Karg and Schams (1974) reported a positive correlation of day length and basal prolactin levels in cattle. Relkin (1972) showed that changes in light: dark ratios for rats altered pituitary prolactin content and plasma prolactin concentrations. This effect is seen after only 4 to 8 hr. of exposure to different lighting regimes. Photoperiod may be a factor influencing pituitary prolactin secretion. Under Florida conditions seasonal temperature changes also are correlated with increasing periods of day length (Gwazdauskas, unpublished observations). Thus, temperature

and photoperiod effects are confounded in evaluating seasonal effects on plasma prolactin concentrations. Under controlled environmental conditions of the present study, temperature seemed unimportant in eliciting a major change in prolactin secretion.

Figure 7 shows the plasma corticoid response when data were synchronized to the LH peak. Statistical analysis revealed no differences ($P>.10$) between treatment means (Appendix, tables 4 and 10). Furthermore, we were unable to detect any individual treatment time trends after looking at regressions up to the 5th order. After accounting for corticoid variability due to treatment, heifers within treatment and time trends (up to the 5th order), there was a 65% C.V. for plasma corticoid concentrations. Our data, with blood samples taken at 4 hr. intervals at least 2 days prior to estrus, do not support Miller and Alliston's (1974a) finding of increased corticoids early the day of estrus (twice daily sampling). Nor does it support a report which showed lower plasma corticoids in dairy cows during summer months in Arizona (Stott and Wiersma, 1973). However, Arizona climatic conditions of high temperature and low humidity may be different from our study with high humidity and high temperature. Our results show numerous episodic peaks during the day. Wagner and Oxenreider (1972) also reported episodic peaks of plasma corticoids when measured at 30 min. intervals throughout the day. They also noted diurnal corticoid variation, but we were unable to detect any time of day differences ($P>.10$) when data were analysed at 4 hr. intervals. Due to large variability in plasma corticoid levels, a large treatment difference in corticoid concentrations would be needed to detect a

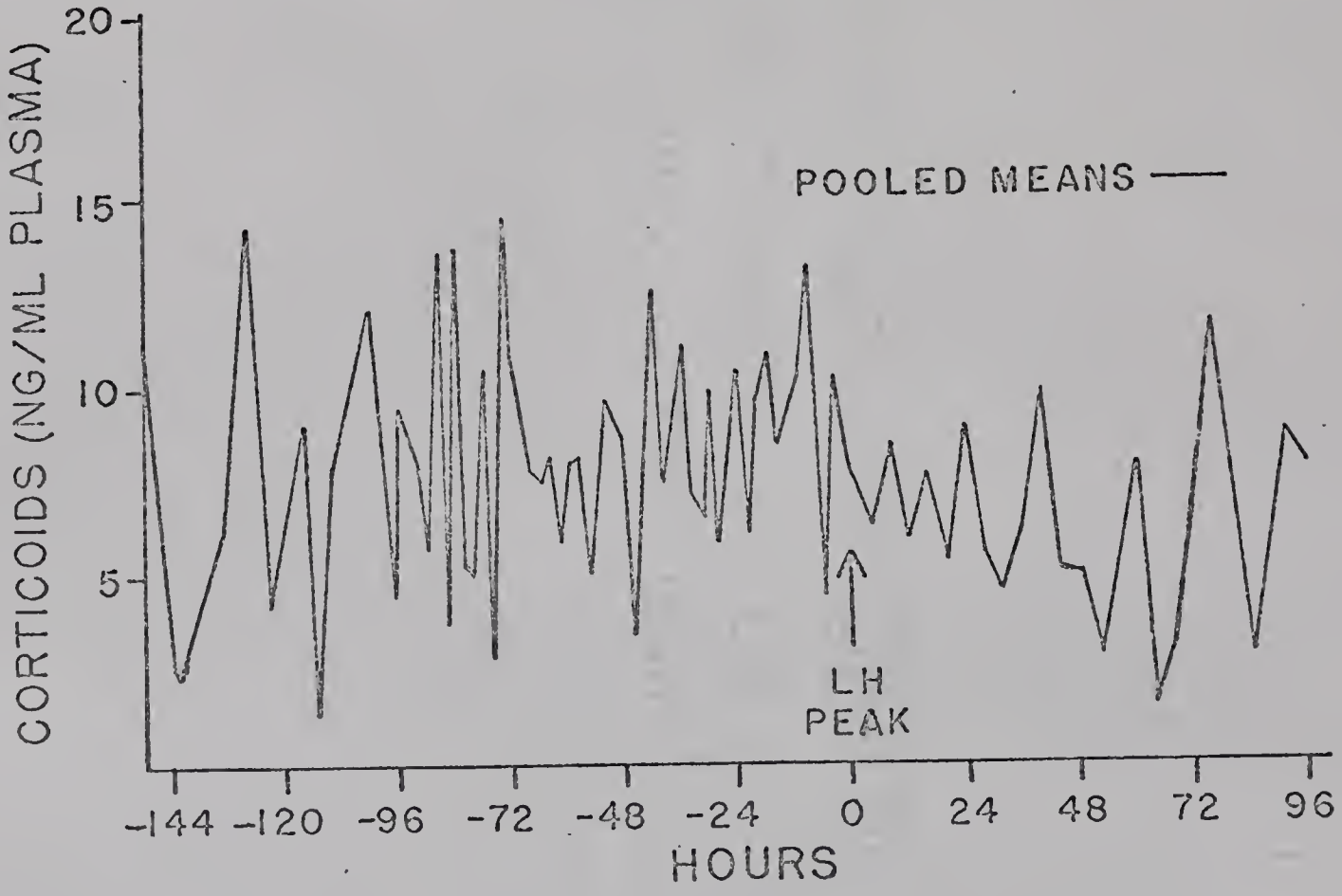

FIGURE 7. SEQUENTIAL CHANGES IN PLASMA CORTICOIDS SYNCHRONIZED TO THE TIME OF THE LH PEAK USING POOLED MEANS OF HEIFERS AT 21.3 C AND 32.0 C.

significant difference. We failed to detect any differences in plasma corticoid associated with estrus, ovulation or heat stress when monitoring plasma concentrations at 4 hr. intervals.

When time was removed from the model and each hormone considered as a dependent variable, plasma LH had a negative association with progestins ($r = -.14$, $P<.05$; table 2). Progestins also were negatively related to estradiol ($r = -.14$, $P<.05$). In the overall model LH was positively related to estradiol ($r = .45$, $P<.01$). These observations are consistent with findings of Chenault et al. (1975) and support their hypothesis that progestins may be inhibiting estradiol biosynthesis and LH release. The significant relationship between plasma corticoids and prolactin ($r = .22$, $P<.01$) may be related to the stress response of both hormones (Gwazdauskas, Thatcher and Wilcox, 1972; Koprowski and Tucker, 1973).

Increases in plasma and blood volumes due to heat stress have been reported in cattle (Bianca, 1965), and chronic exposure to heat resulted in decreased hematocrit. Changes in plasma electrolyte concentrations due to thermal stress are reported to be slight (T. N. Wegner, personal communication). Cattle in tropical areas had a higher body water content in summer than in winter months (Thompson, 1973). Such factors as plasma volume and dilution may influence interpretation of hormonal responses to a controlled heat stress. For example, a difference in plasma corticoid concentration was not detected in our study. However, a heat stress induced increase may have been undetectable due to a possible plasma dilution response of these heifers. Conversely, a decrease in estradiol concentration may have occurred

due to plasma dilution and not necessarily decreased secretion. Such criticisms apply to all other hormonal responses in this study. Thus, it was of interest to measure plasma total protein concentration and plasma osmolality to determine if a possible plasma dilution had occurred.

Plasma samples from 8 to 96 hr. after the LH peak and having less than 3 pg/ml estradiol were pooled within heifer for evaluation. There was no difference ($P>.10$) in total protein concentration or plasma osmolality between heifers at 21.3 C and 32.0 C (table 3). These results suggest that no appreciable plasma dilution had occurred. However, we have no measurement of total plasma volume of heifers for this study.

A Corticosteroid Binding Globulin (CBG) of plasma has been reported for various species (Seal and Doe, 1965) and also is present in the bovine (Lindner, 1964). Such a protein acts as a corticoid carrier molecule through the blood. Although total plasma protein concentration (table 3) did not vary between treatments, certain alterations of protein composition may have occurred. Although plasma corticoid concentrations did not differ between treatments, their potential biological effectiveness would be appreciably altered if the concentration of plasma CBG differed.

Utilizing the procedure of Pegg and Keane (1969), the association constant (K_a) and cortisol binding capacity of CBG were determined on pooled samples (within heifer) of each experimental heifer. The association constant did not vary due to treatment ($P>.10$; table 3). An average experimental K_a of 1.86×10^7 M^{-1} was indicative of a protein with an intermediate affinity for the cortisol ligand. It is a protein

Table 3. Physical characteristics of plasma in heifers at 21.3 C and 32.0 C.

	21.3 C	32.0 C
Protein (mg/ml)	76.38 ± 8.52[a]	75.50 ± 9.05
Osmolality (milliosmoles/kg H_2O)	259.65 ± 11.65	268.70 ± 10.41
Corticoids (ng/ml)	6.7 ± 1.2	5.9 ± 1.0
Cortisol Binding Capacity (ng/ml)	118.93 ± 48.02	55.86 ± 11.19*
Association Constant (K_a X 10^7 M^{-1})	1.52 ± .72	2.20 ± .69

[a] ($\bar{X}$ ± SD)
*($P<.05$)

with an association constant higher than a low affinity protein such as human serum albumin (K_a = 1 X 10^4 M^{-1}) but lower than the K_a for a tissue receptor protein such as the cortisol mammary gland receptor (K_a = 5 X 10^8 M^{-1}; Tucker, Larson and Gorski, 1971). It was not expected that thermal stress would alter the physical chemical properties of the CBG protein (K_a) but perhaps may alter the amount (capacity) of CBG per ml of plasma. Indeed there was a significant difference ($P<.05$) in cortisol binding capacity (ng/ml) between treatments (table 3). Thus under experimental conditions for quantifying cortisol binding capacity at 4 C, plasma of heat stressed heifers had a 53% lower capacity to bind cortisol. This suggested that under such conditions, plasma from hyperthermic heifers contained a decreased concentration of CBG. Therefore, under environmental temperatures of 32.0 C at a body temperature of 40.24 C both the concentration of plasma CBG and cortisol bound CBG (product) would be less if the rate constant for the forward reaction was not different at an elevated body temperature of 1.5 C (table 1). With this reasoning, heifers exposed to a thermal stress characteristic of our experiment would have a greater percent free cortisol compared to CBG bound cortisol at a constant total cortisol concentration. As previously described, total plasma corticoid concentrations did not vary between treatments (6.7 compared to 5.9 ng/ml; table 3).

Under other stressful conditions a lowered corticoid binding capacity has been reported for various species. In human burn patients a slightly lowered cortisol binding capacity has been reported (Mortensen et al., 1972), in which decreased capacity was inversely related to burn area. By analogy, lactation can be considered a stress in the sense

that reproductive efficiency is lower during this period. Lactation inhibits the onset of estrous cycling in rats nursing 6 or 12 pups compared to post-parturient rats which do not lactate (Tucker and Thatcher, 1968). Early weaning of calves from their dams increased the occurrence of estrus and increased pregnancy rates in beef and dairy cattle (Laster, Glimp and Gregory, 1973). Troconiz (1973) has reviewed the cystic ovary condition in dairy cattle. High milk producing cows had a greater incidence of cystic ovaries and therefore a greater frequency of reproductive problems. In rats nursing 12 pups, CBG activity was lower in comparison to rats nursing only four pups (Westphal, 1970). Such a nursing intensity will delay occurrence of normal estrous cycles (Tucker and Thatcher, 1968). Thus under conditions of lactational stress (relative to reproductive performance) CBG activity was depressed,

The liver is the reported source of CBG (Guyton, 1966) and the thyroid gland is reported to exert a controlling influence on CBG activity. Gala and Westphal (1966) showed that TSH stimulated CBG activity in hypophysectomized rats and was primarily responsible for regulation of CBG levels. In cattle under conditions of high environmental temperatures, thyroid activity was depressed (Johnson and Yousef, 1966). If the hormonal control of CBG production is grossly comparable between rats and cattle, then lower CBG binding capacity of heifers detected in our study would be expected.

Hypothyroid patients have a slower turnover of cortisol. Both bound and free steroid fraction disappearance rates were slower than normal or hyperthyroid patients (Beisel et al., 1964). In our study the amount of free hormone would have a greater biological role in the

heat stress group due to the lower binding capacity. This may result in a lower level of ACTH secretion due to greater negative feedback inhibition. In the bovine, corticoid turnover rates were depressed during chronic heat stress (Christison and Johnson, 1972). This also suggests a longer biological life for the circulating corticoid allowing a greater ACTH negative feedback since less corticoid is also bound to transcortin. However, the amount of free cortisol in our study, estimated by extrapolation at 4 C, was not different ($P>.10$) between the 21.3 C heifers (1.39 ng/ml) and 32.0 C heifers (1.43 ng/ml).

Clarification is needed in this area as to the physiological role of bound and free steroids because of conflicting reports between species and stress situations. Our finding that corticoids were not elevated during chronic heat stress, irrespective of the binding capacities, may be advantageous to the cow in that heat production has been shown to increase 30 to 40% at 35 C when hydrocortisone acetate was administered (Yousef and Johnson, 1967).

In the second phase of the experiment, 8 days following ovulation in the last heifer, 200 IU ACTH was injected, IV, into 10 heifers. The ACTH was given while heifers were in the luteal phase of the estrous cycle or at a time when a progesterone increases in peripheral plasma due to ACTH injection (Gwazdauskas, Thatcher and Wilcox, 1972; Wagner, Strohbehn and Harris, 1972) may not have a detrimental effect on the developing embryo (Johnsson *et al.*, 1974). Figure 8 shows the corticoid response curves following ACTH injection. The 32.0 C group responded with significantly lower ($P<.10$) corticoid concentrations. The 6th order regression curves were not parallel ($P<.01$) suggesting that the

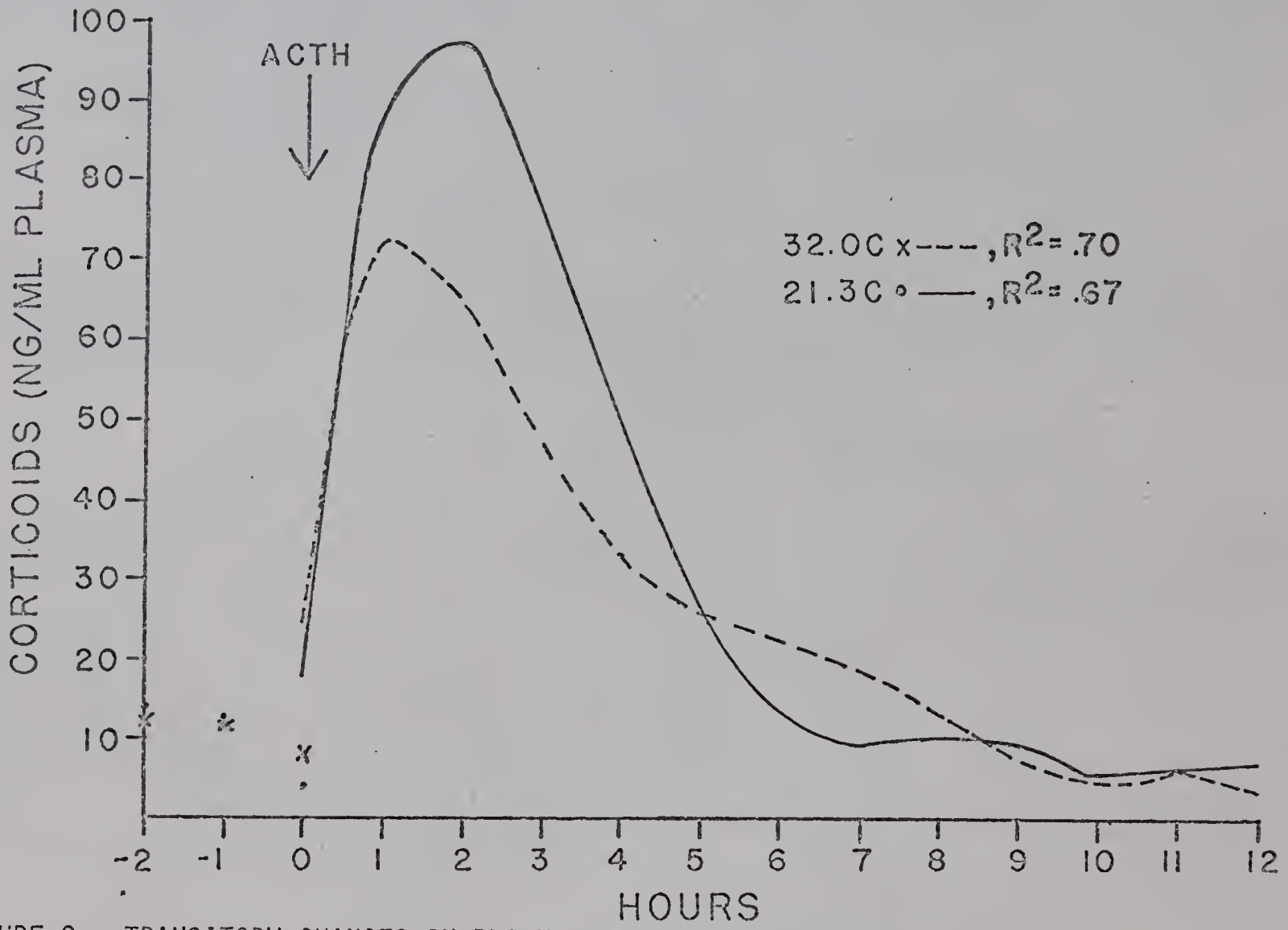

FIGURE 8. TRANSITORY CHANGES IN PLASMA CORTICOIDS FOLLOWING INJECTION OF 200 IU ACTH IN HEIFERS AT 21.3 C OR 32.0 C.

hot group response was earlier to reach a peak (75 min. compared to 105 min.), had a lower magnitude (73.5 compared to 100.2 ng/ml corticoid) and was of shorter duration (4 hr. compared to 5 hr.; Appendix, table 11). This response is comparable to that reported by Shayanfar (1973) in which lactating cows exposed to environmental temperatures above 21.1 C responded the same way. The cool heifer response was best described by: $\hat{Y}$ (corticoids, ng/ml) = $-521.387 + 4648.999X - 12253.643X^2 + 13609.684X^3 - 5942.539X^4 + 4.326X^5 + 464.541X^6$ ($P<.01$), whereas the hot heifer response was best characterized by $\hat{Y} = -613.342 + 6525.433X - 23576.092X^2 + 41551.725X^3 - 38756.632X^4 + 18359.263X^5 - 3477.023X^6$ ($P<.01$).

The apparent reduced ability of the adrenal to secrete and/or synthesize corticoids following ACTH stimulation during heat stress may be related to a chronic lower level of endogenous ACTH secretion. The lower plasma cortisol binding capacity in the 32.0 C heifers may provide a greater amount of free corticoid to exert a feedback inhibition on endogenous ACTH secretion. In addition, there is also a lower level of corticoid turnover and secretion during chronic heat stress (Christison and Johnson, 1972). As a result the degree of chronic endogenous ACTH secretion may be less, causing a reduction of responsive adreno-cortical tissue. These conditions may result in a lower adrenal corticoid increase in response to a pharmacological challenge with ACTH. A reduced level of adrenal function during heat stress would be advantageous to the animal calorigenically. Corticoid secretion did not appear to be higher in the heat stressed group since resting corticoid levels were not greater than controls. However, a possible decreased adrenal secretion rate was not reflected by a lower plasma corticoid concentration. It was not until

a response to ACTH was evaluated that adrenocortical function appeared to be depressed.

To determine the significance of this apparent reduction in adrenal responsiveness to ACTH due to hyperthermia plasma ACTH levels need to be determined in the bovine under different physiological stress situations. Other possibilities include determining effects of stress on ACTH receptors, more definitive studies on corticoid-CBG binding properties in relation to thermal stress and possible steroidogenic-enzyme alterations in the adrenal.

Pre-ACTH plasma progestin and corticoid concentrations were tested to detect differences in levels due to heat stress and pregnancy status (Appendix, tables 11 and 12). The analyses include temperature, pregnancy status and heifers nested in temperature-pregnancy status. There were no statistically significant differences ($P>.10$) either in hormone concentrations due to temperature or pregnancy status. However, significant among animal variability ($P<.05$) was found in progestin levels. These results agree with the pre-$PGF_{2\alpha}$ treatment hormonal values in the first phase of this experiment (Page 30). Our observations conflict with a summer seasonal depression in corticoid and progestin concentrations reported by Stott and Wiersma (1973). The present study also did not confirm their finding of higher progestins in fertile cows on day 15 of pregnancy or the estrous cycle. This period of corpus luteum function is comparable to our study (heifers were between estrous cycle days 9-13).

In summary, environmental treatment of 32.0 C evoked a 1.49 C increase in rectal temperature and a 3 to 4 C increase in skin

temperatures. The time durations between $PGF_{2\alpha}$ injection and the LH peak and the period between $PGF_{2\alpha}$ and ovulation were not different ($P>.10$) between treatments. Length of estrus was shorter ($P<.10$) for the heat stressed heifers. Two of four heifers inseminated in the 21.3 C chamber were pregnant at 40 days compared to none of five in the 32.0 C chamber. Thus, the environmental condition did affect body temperature, duration of estrus and overall fertility.

Preinjection plasma samples showed no differences ($P>.10$) in any of the hormonal measurements due to the main effect of temperature. Average progestin concentration between treatments was not different ($P>.10$). However the 5th order response curves were not parallel ($P<.01$) indicating a different time response between treatments. Progestin concentrations declined in a similar manner in both groups following $PGF_{2\alpha}$ injection. Heifers in the 21.3 C group, on the average, had a LH surge about 24 hr. later than heifers in the 32.0 C group. This 24 hr. time lag would account for the difference in time responses when data were synchronized to time of LH peak. Mean estradiol concentrations were significantly ($P<.10$) lower in the heat stressed heifers. The lower plasma estradiol may have contributed to the shorter estrous periods seen in the 32.0 C heifers. However, these lower concentrations of estradiol were adequate enough to elicit estrous behavior and trigger LH release causing a subsequent ovulation.

Estrone showed no apparent association with the onset of estrus or LH peak when the data were synchronized to the time of the LH peak. There was a significant elevation ($P<.05$) of estrone due to heat stress but there was no evidence that estrone time trends following $PGF_{2\alpha}$ were

not parallel (P>.10) suggesting that in both treatments estrone followed a similar decline postinjection. No significant differences (P>.10) were found in mean LH concentrations between heifers at 21.3 C or 32.0 C. Preovulatory peak LH concentrations were 32.2 ng/ml and 33.2 ng/ml plasma for the 21.3 C and 32.0 C heifers, respectively. All animals had a preovulatory LH surge, suggesting that hyperthermia did not prevent the triggering mechanism for LH release.

There was no change in prolactin associated with estrus or the LH peak, therefore prolactin was analyzed relative to time of $PGF_{2\alpha}$ injection. Mean prolactin concentrations were not different between treatments (P>.10). The 4th order time curves were not parallel (P<.005). Heifers in the 21.3 C chamber had a decline in plasma prolactin after the initial sampling as compared to increased prolactin concentrations in the 32.0 C heifers during this early blood sampling period. The summer seasonal increase in plasma prolactin reported by various researchers may be more related to photoperiod effects. There was no difference (P>.10) between treatment means in plasma corticoid concentrations. Furthermore, we were unable to detect any individual treatment time trends after looking at regressions up to the 5th order. Plasma corticoid C.V. was 65% after accounting for variability due to treatment, heifers within treatment and time trends up to the 5th order.

In an attempt to determine if plasma dilution may have occurred, total protein concentration and osmolality were measured. There was no difference (P>.10) in total protein concentration or osmolality between treatment groups. However, no measurement of total plasma volume was made. Cortisol binding capacity of CBG and its association constants

(K_a) were determined. The affinity (K_a) of cortisol for CBG was not different between treatments (P>.10); however, the binding capacity of CBG for cortisol was significantly (P<.05) reduced in the 32.0 C heifers. This observation suggested that under experimental conditions (4 C) for determining the binding capacity of cortisol, the hyperthermic heifers may have had a decreased concentration of CBG.

ACTH (200 IU) was injected, IV, into 10 heifers. The 32.0 C heifers responded with a significantly lower (P<.10) corticoid concentration. The 6th order regression response curves were not parallel (P<.01) suggesting that the hot group response was earlier to reach a peak (75 min. compared to 105 min.), had a lower magnitude (73.5 compared to 100.2 ng/ml corticoids) and was of shorter duration (4 hr. compared to 5 hr.). Adrenal responsiveness was significantly less in heifers maintained at 32 C.

Results of this experiment show only subtle thermal effects on plasma concentrations of estradiol and estrone and no effects on LH, progestins, corticoids and prolactin. Apart from possible hormonal involvement with duration of estrus, heat stress does not appear to affect the hormonal milieu associated with corpus luteum regression, follicle growth and ovulation. The significance of possible lowered adrenal response in hot environments may be related to a state of lowered heat production. Since corticoids are known to be calorigenic (Yousef and Johnson, 1967) a lowered adrenal responsiveness in hyperthermic heifers might be physiologically advantageous.

The experiment described in this section has not specifically considered the possible environmental and hormonal effects on uterine

temperature. It was of prime importance to characterize uterine thermal changes during the period of luteal regression, follicle growth and ovulation under conditions of a mild heat stress, and to document possible estrogen induced uterine thermal changes.

SECTION III

EXPERIMENT I: THERMAL CHANGES OF THE BOVINE UTERUS FOLLOWING ADMINISTRATION OF ESTRADIOL-17β

Introduction

The first experiment (Section II) indicated that a thermal stress increased body temperature, suppressed fertility and caused a slight decrease in endogenous estradiol secretion. Furthermore, we reported previously that uterine temperatures both on day of and day after insemination were inversely related to fertility (Gwazdauskas, Thatcher and Wilcox, 1973). This directly indicated that temperature of the uterus was closely associated with fertility.

Other factors in addition to environmental temperature may influence uterine temperatures. For example estrogen administration was shown to increase uterine blood flow in sheep (Huckabee et al., 1970; Greiss and Anderson, 1970; Rosenfeld et al., 1973; and Resnik et al., 1974). This uterine hyperemia may have caused heat to be dissipated from the uterus, thus cooling the uterine cavity (Abrams et al., 1970a). Uterine blood flow changes in sheep were monitored following estrogen injections by looking at differences in temperature between the uterus and aorta. A rise in blood flow rate resulted in a lower uterine temperature (Abrams et al., 1970a).

Although uterine temperature and estrogen relationships have been found in sheep, this phenomena has not been examined in the bovine. Objectives of this study were to determine if uterine-aortic temperature differences exist in the bovine, and if such differences change following injection of Estradiol-17β.

Materials and Methods

Thermocouple Preparation and Calibration

Lengths of 36 gauge, nylon coated, copper constantan wire (Revere Corp., Wallingford, Conn.) were pulled through polyvinyl tubing (V5-V7; Bolab Inc., Derry, N. H.) for measurements of uterine and blood temperatures. The terminal thermojunctions to be placed in the saphenous artery then were pulled through a larger polyvinyl tube (V-12) for additional support. The ends of all thermojunctions were heat-sealed in the polyvinyl by pushing them through a siliconized, narrowbore glass tubing which was being heated on a soldering iron. After sealing, ends were coated with liquid tygon (U. S. Stoneware Co.). Stranded, untinned copper extension wires (Leads and Northrup, #27-32-36, Philadelphia, Pa.) were soldered to divided copper wires leading to the thermojunctions. All extension wires led either to a millivolt potentiometer (#8686, Leads and Northrup, Philadelphia, Pa.; limits of error of recording system $\pm$ .075 C) or to a strip chart recorder (Hewlett-Packard, M 7100B; limits of error of recording system $\pm$ .03 C). Most, but not all of the potentials from the aortic-ice water thermocouple were suppressed by known amounts before being amplified and recorded.

Calibration of the thermocouples was made routinely by use of a Bureau of Standards Certified Thermometer in a well-stirred, insulated water bath held at intervals between 36 to 40 C. The thermocouple readings were 0.05 to 0.075 C above the certified thermometer reading, so all data collected were corrected for these constants.

Surgical Techniques and Experimental Protocol

Four 2-year-old heifers with histories of regular estrous cycles were used in these experiments. Prior to surgery, heifers were placed on a 48 to 72 hr. feed and water fast. Heifers were anesthetized with 2 to 4 g sodium thiopental (Abbott Laboratories, North Chicago, Ill.) dissolved in saline (2 g/20 ml) while standing and restrained. They were placed onto a portable operating table, tracheotomized and maintained under surgical anesthesia with methoxyfluorane (Pitman-Moore, Washington Crossing, N. J.). After removal of hair, the abdominal and inguinal regions were scrubbed thoroughly with germicidal soap and rinsed with 70% alcohol.

A 15 cm longitudinal midventral incision was made through the abdominal wall at the cranial margin of the mammary gland. A sharpened stainless steel cannula was carried into the abdominal cavity through this midventral incision and pressed through the abdominal wall in the flank area. All thermojunctions and approximately 2.5 m of extension wires were drawn through the cannula leaving the remainder of the 3 m of extension wire and connectors coiled up in a canvas pack. The cannula was removed from the abdominal cavity by sliding it over the thermojunctions and withdrawing it through the midline incision. The

pack subsequently was attached to the flank with one or two stainless steel pins passed through a flap of skin.

The uterus was elevated so that the junction of the uterine horns with the uterine body could be visualized. Using small scissors and straight forceps, a 3 to 4 cm tunnel was made under the serosa in the medial aspect of one uterine horn about 1 cm from the bifurcation. A thermojunction was inserted into this tunnel and tied in place with 000 silk thread. The extension wires were secured by two to three additional ties through the serosa along the uterine horn.

Thermojunctions for aortic blood temperatures were routed through the midventral incision, tunneled under the skin to the inguinal area where the saphenous artery was exposed. These thermojunctions then were inserted into the saphenous artery, passed 70 to 75 cm upward to the abdominal aorta and extension wires fixed with silk suture at the point of entry into the vessel. Incisions were closed in layers. Thermocouple placements were confirmed prior to their surgical removal 7 to 10 days after completion of the experiment.

Twenty-four hr. prior to intravenous (IV) injection either of 3 mg Estradiol-17β (Progynon-Schering Corp., Bloomfield, N. J.) or 12 ml of .9% sterile saline, heifers were fitted with polyvinyl catheters (V-7) by jugular venipuncture. Catheters were filled with heparin solution (15 U/ml of .9% saline), capped with a brad and the external catheter placed in an adhesive tape pouch glued to the neck with branding cement (Electro Cote Co., Minneapolis, Minn.).

On the day of injection heifers were placed in a stanchion barn on rubber comfort mats at least 2 hr. prior to recording temperatures.

Each of the four heifers received an estradiol injection, and two of the heifers also received two saline injections each. Thus there were a total of four estradiol and four saline experiments. All heifers received treatment during the luteal phase of the cycle. Recordings were made from the millivolt potentiometer at 15-min. intervals beginning 1 hr. prior to IV injection either of Estradiol-17β or saline and ending 6 hr. after the injections. A pre-experimental control period of 1 hr. was used to determine a steady state level of uterine temperature. Repeatability of triplicate measurements at each time was 0.92 for aortic temperature (mV) with a C.V. of 0.07% (n=66). Repeatability and C.V. for $\Delta T_{uterus\text{-}aorta}$ (μV) were 0.99 and 6.44%, respectively.

Initially, an additional thermocouple was placed in the uterine lumen as well as in the uterine serosa. Prior to and following estrogen injection the temperatures at both reference points were identical. To avoid any possible complication due to presence of an intrauterine object, all subsequent animals were fitted only with a uterine serosa thermojunction. In a separate experiment, temperatures were recorded continuously before and after an injection of Estradiol-17β. The major statistical technique to analyse time changes was least squares as described by Harvey (1960). Statistical models were selected based on tests of significance of the higher order terms in the regression analyses and visual appraisal of the graphs.

Results and Discussion

The uterine and aortic temperature response following intravenous injection of 12 ml saline is shown in figure 9. The slight increase in

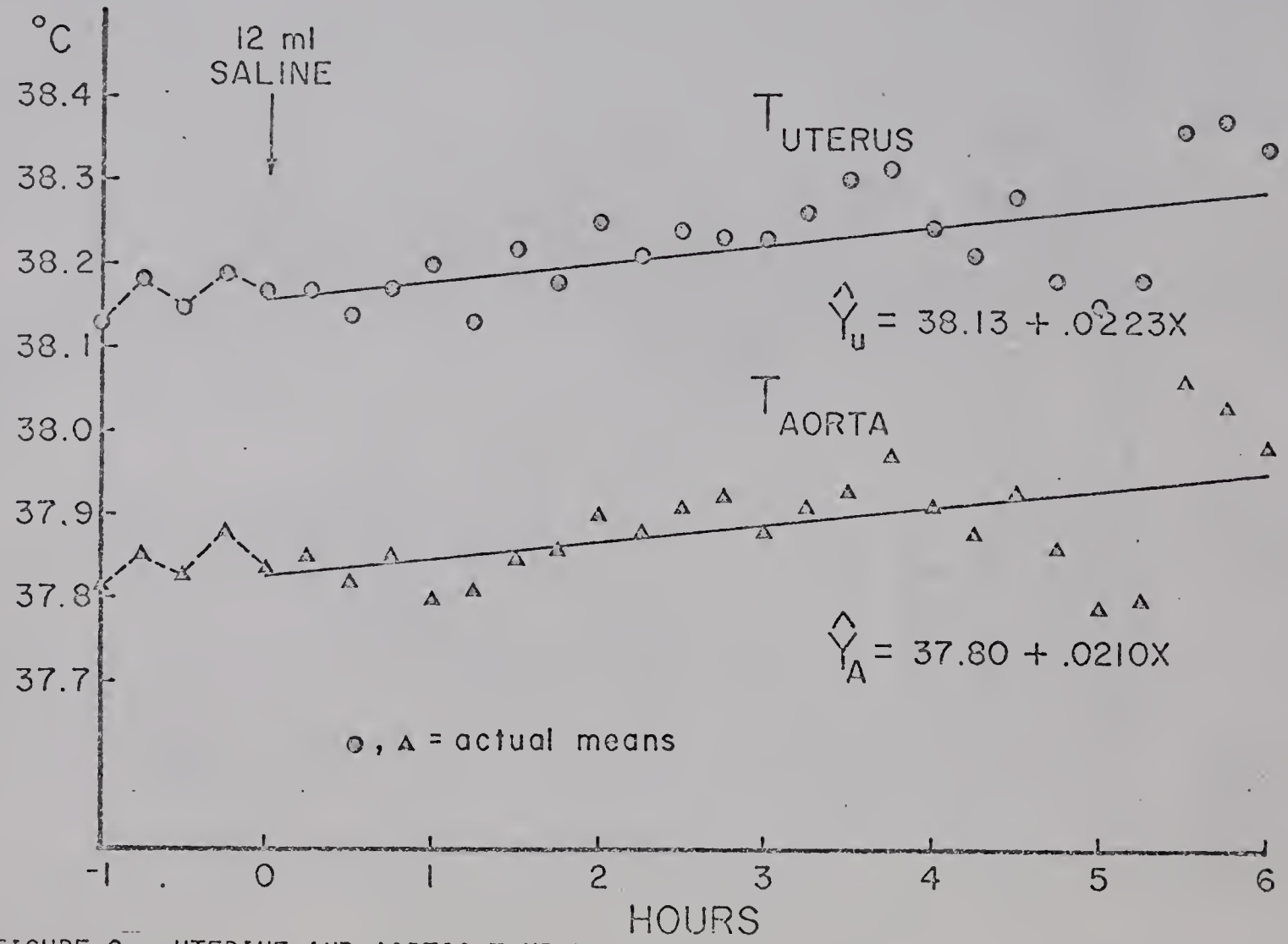

FIGURE 9. UTERINE AND AORTIC TEMPERATURE PRIOR TO AND FOLLOWING IV INJECTION OF 12 ML STERILE SALINE.

both mean temperatures (~ 0.2 C) which occurred during the 7 hr. experiment may be related to the normal rhythmic rise in body temperature in cattle during the day (Bianca, 1968). The greater variability in both temperatures 4 to 6 hr. after saline injection could have been because of blood temperature changes induced by some restlessness due to long confinement in the stanchions. In spite of these changes in uterine and aortic temperatures, temperature differences between the two were quite stable during the experiment, indicating that the ratio between uterine heat production and uterine heat loss had remained unchanged. Relationships of time (X) and uterine (Y_u) and aortic (Y_a) temperatures are shown in figure 9. There was no evidence of curvilinearity; fitting the two equations accounted for 36 and 37% of the within-heifer variability in Y_u and Y_a, respectively. There was no evidence that the two slopes were not parallel, which suggested that the saline vehicle had no depressive effect either on uterine or aortic temperature.

Effects of Estradiol-17β on uterine and aortic temperatures are illustrated in figure 10. The initial fall in uterine temperature of slightly more than 0.3 C compares favorably with the response noted previously in sheep (Abrams et al., 1970a). The slight rise in uterine temperature between 4 to 6 hr. post injection was undoubtedly due to the rise in blood temperatures as noted in control experiments. Uterine changes were curvilinear ($P<.01$) as indicated by the equation ($R^2 = 0.18$). A significant quadratic equation ($P<.01$; $R^2 = 0.04$) best describes the aortic temperature response. Why aortic temperature fell initially is not known. Increased respiratory evaporative heat loss or sweating may have been responsible. Estrogens are known to be potent

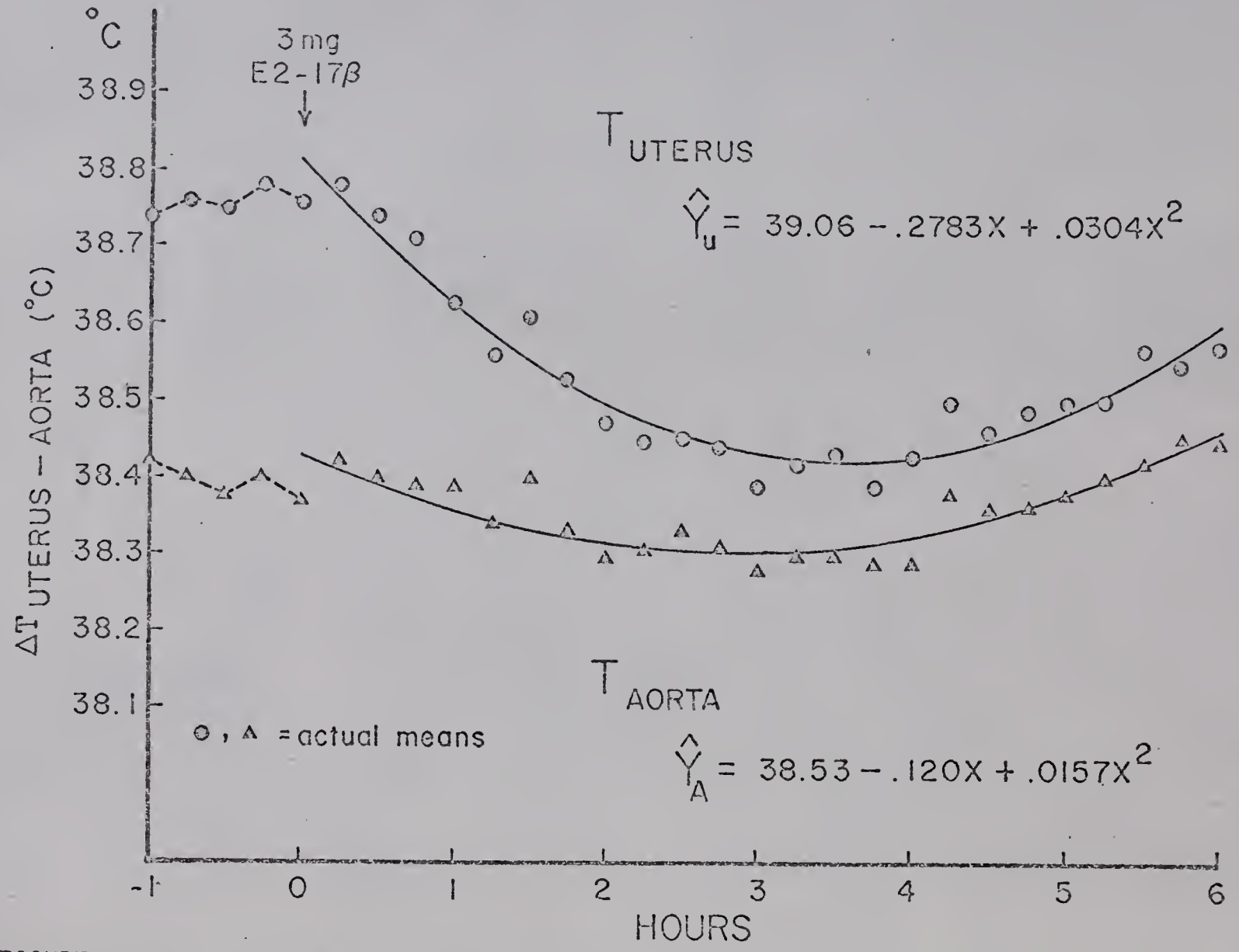

FIGURE 10. UTERINE AND AORTIC TEMPERATURE PRIOR TO AND FOLLOWING IV INJECTION OF 3 MG ESTRADIOL-17β.

vasodilators of skin blood vessels (Reynolds and Foster, 1940), and to the extent that heat loss was promoted by this increased skin blood flow, a lowered temperature may result. One may propose that estrogens could have had a subtle effect of the thermoregulatory "set point" (Hammel et al., 1963) which resulted in activating one or more heat loss mechanisms. However, the decrease in aortic temperature was only about .1 C.

When the difference in temperature between uterine serosa and aorta was examined the result of Estradiol-17β administration was obvious (figure 11). The decrease in $\Delta T_{uterus\text{-}aorta}$ ($\Delta T_{u\text{-}a}$) of 0.25 C was described by a highly significant ($P<.01$) curvilinear trend over time. The $\Delta T_{u\text{-}a}$ began to plateau at approximately 2.5 hr. post estrogen injection and remained depressed for the duration of the recording period, although both uterine and aortic temperature started to rise 4 to 5 hr. post-injection. There was no significant change ($P>.10$) in $\Delta T_{u\text{-}a}$ following saline injection.

Figure 12 is a plot in 30 sec. intervals taken from a continuous recording of temperatures of one heifer prior to and following Estradiol-17β injection. In this animal the estrogen effect on uterine temperature was noted within 1 hr. Rapid oscillations in temperature of the uterine tracing were considerably dampened by the heat capacity of the uterine tissue.

A consistent finding in the estrogen experiments was the decrease in the temperature difference between the uterus, as represented by the subserosal temperature and the blood of the abdominal aorta. Such a decrease in ΔT could occur as a result of a lowered rate of uterine

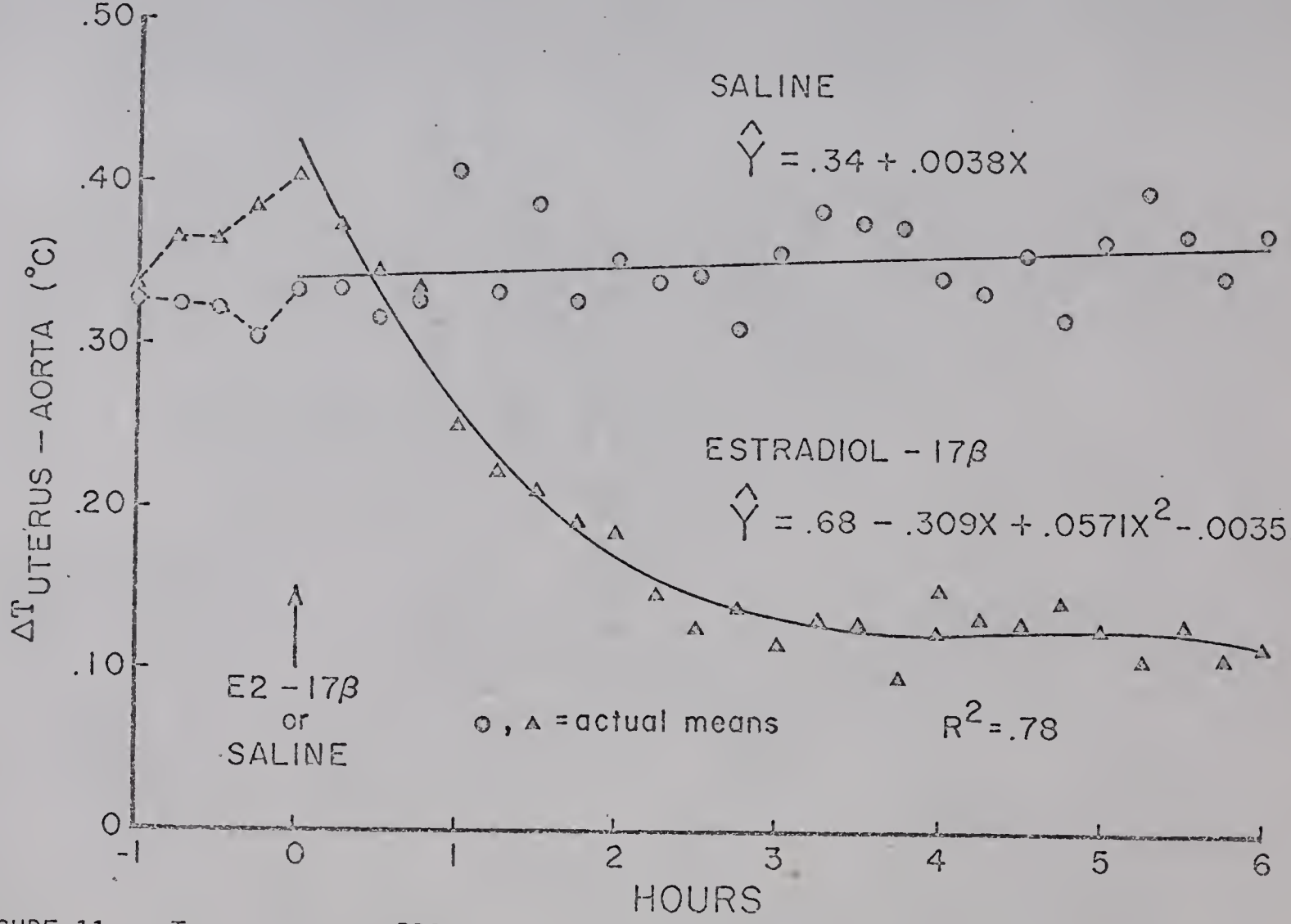

FIGURE 11. $\Delta T_{uterus-aorta}$ PRIOR TO AND FOLLOWING EITHER 12 ML SALINE OR 3 MG ESTRADIOL-17β.

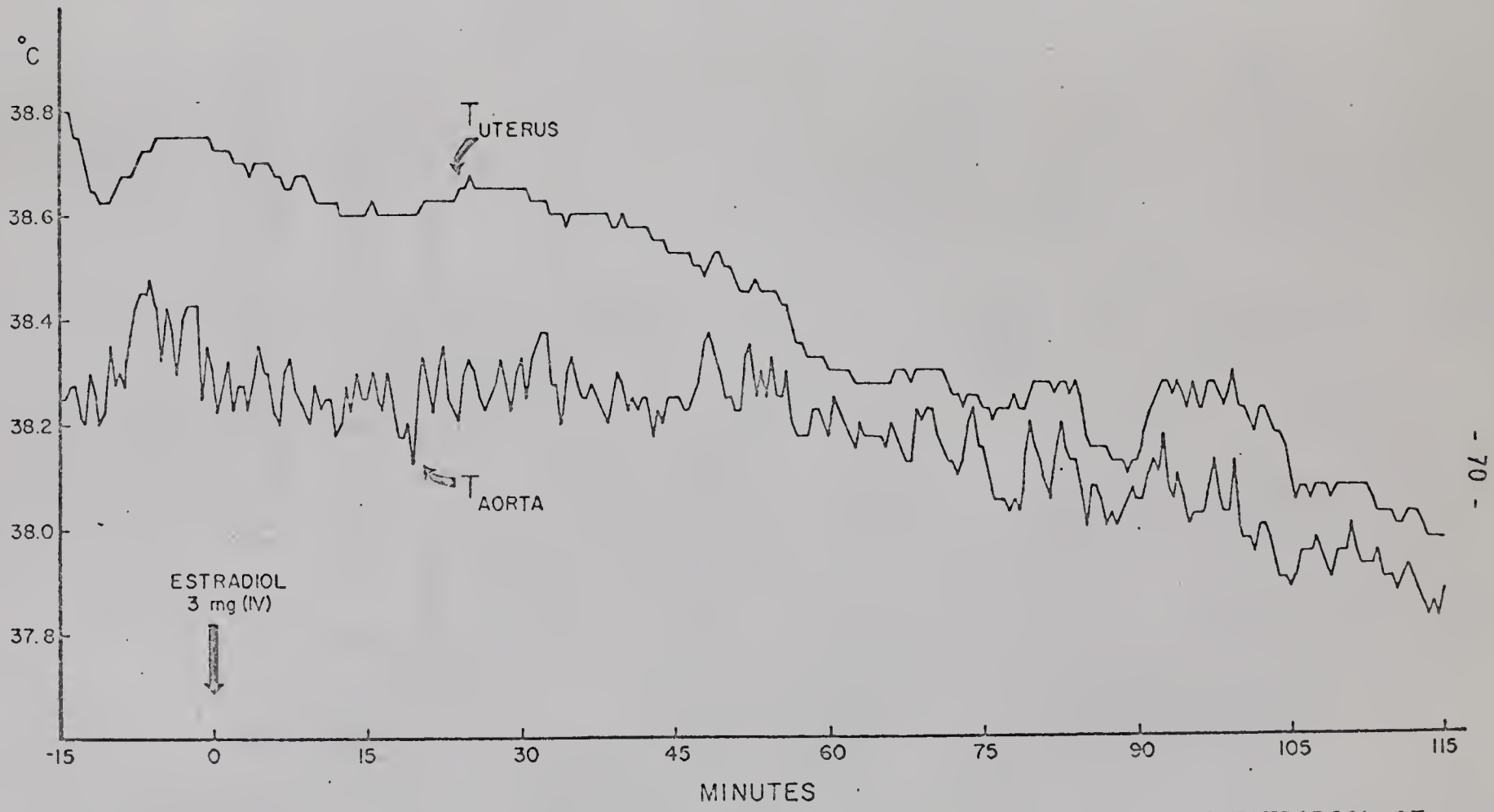

FIGURE 12. UTERINE AND AORTIC TEMPERATURE PRIOR TO AND AFTER INJECTION OF ESTRADIOL-17β FROM CONTINUOUS RECORDING.

heat production, a possibility which appears remote in view of the many cellular metabolic activities induced by estrogens (Talwar and Segal, 1971). A more reasonable explanation for the lowered ΔT_{u-a} is the augmented rate of heat loss resulting from the marked estrogen induced elevation in uterine blood flow. Endogenous estrogens released during the estrous cycle in ewes are known to be associated with elevated uterine blood flow rate (Greiss and Anderson, 1970) and increased vaginal blood flow as inferred from a significant rise in vaginal thermal conductance in cattle (Abrams et al., 1973). Thus, there is reason to believe that comparable cyclic, blood flow-induced changes in temperature of the reproductive tract may occur during the estrous cycle in the bovine. High uterine temperatures at the time of artificial insemination are associated with diminished fertility (Gwazdauskas, Thatcher and Wilcox, 1973). Elevated environmental temperature is thought to suppress fertility by acting directly on the developing embryo and/or through altering maternal endocrine function (Vincent, 1972).

Findings in the first experiment indicated that plasma estradiol of heat stressed heifers was lower during the pre-estrous period. Results of the present study indicate that a pharmacological injection of Estradiol-17β can significantly decrease uterine temperatures. In the final experiment, attempts were made to evaluate changes in uterine temperature during the period of luteal regression (decreasing progesterone), follicle growth (increasing estradiol) and ovulation under conditions of a mild heat stress.

EXPERIMENT 2: THERMAL CHANGES IN THE BOVINE UTERUS FOLLOWING $PGF_{2\alpha}$ INJECTION THROUGH ESTRUS AND OVULATION

Introduction

The first experiment (Section II) indicated that a thermal stress increased body temperature, suppressed fertility and caused a slight decrease in endogenous estradiol secretion. Next, an effect of exogenous Estradiol-17β on uterine temperature was documented. In this final experiment, estrus was synchronized by $PGF_{2\alpha}$ and an attempt was made to evaluate changes in uterine temperature and aortic blood temperature with plasma estradiol and LH under conditions of mild heat stress. Such an experiment would closely mimic responses of animals under normal field conditions and provide additional insight into factors controlling uterine temperature under conditions of poor reproductive efficiency.

Materials and Methods

Thermocouple preparation and calibration were the same as described in the previous experiment with the exception that all thermocouples were made in triplicate for each location. During the experiment, extension wires led to a recording potentiometer (9835 A, D-C Microvolt Amplifier and Speedomax G, Model S6000 Recorder, Leeds and Northrup, Philadelphia, Pa.; limits of error of the recording system $\pm$ .0125 C). Surgical

techniques were identical except cattle were anesthetized with 3 g sodium thiamylal (Surital-Park Davis, Detroit, Michigan) dissolved in saline (3 g/20 ml) and were maintained under surgical anesthesia with halothane (Fluothane-Ayerst Laboratories, Inc., New York, N. Y.).

Blood samples were collected prior to $PGF_{2\alpha}$ injection (0 hr.), at 6 hr. intervals for 48 hr. and every 4 hr. until 24 hr. after visual detection of estrus. Measurements of LH and estradiol were by methods previously cited. Three cycling first lactation dairy cows between 60 to 90 days postpartum and one cycling heifer were given 30 mg $PGF_{2\alpha}$-Tham Salt (IM). All animals were between days 9 to 15 of the estrous cycle at the time of injection. Each animal had a functional corpus luteum at the time of surgery, 4 to 5 days earlier. At the time of $PGF_{2\alpha}$ injection each animal maintained a uterine-aortic temperature difference (ΔT_{u-a}) greater than .3 C during the previous 2 days and a palpable corpus luteum. A second injection of $PGF_{2\alpha}$ (10 mg) was given to 3 of the 4 cows at 21 hr. after the first injection. This was done to insure complete luteal regression. Cow aortic temperatures and ΔT_{u-a} were monitored continually from 5 hr. prior to the initial $PGF_{2\alpha}$ injection until 24 hr. after the detection of estrus. Twice daily, recordings were temporarily interrupted for 90 min. (0800 and 2000 hr.) for estrous checks and exercise. Temperatures in the heifer were recorded continually for 15 min. prior to $PGF_{2\alpha}$ injection until 6 hr. postinjection.

Rectal palpations were made 2 days postinjection to confirm corpus luteum regression, and again approximately 24 hr. after visual appraisal of estrous behavior to detect occurrence of ovulation.

Thermocouple placement was verified 4 days after estrus by surgical examination of the reproductive tract. At ovariectomy confirmation of luteal regression, ovulation and new corpus luteum formation was verified by disection.

Results and Discussion

Regression of the corpus luteum, as determined by rectal palpation, occurred in all four animals. The three cows, at the time of ovariectomy, had newly formed corpora lutea near the area where the old corpus luteum had regressed. Two of the cows were detected in estrus while the thermocouples remained functional. The usual life span of thermocouples was about 2 weeks. However, due to mechanical failure the thermocouples in one cow lasted only 7 days, and the heifer was not detected in estrus during this period. At the time of ovariectomy and recovery of thermocouples, confirmation of ovulation was made on the basis of a newly formed corpus luteum.

The immediate effects of $PGF_{2\alpha}$ on uterine and aortic temperatures of two cows and ΔT_{u-a} of all four animals are shown in figures 13, 14 and 15. To simplify and assimilate the continuous recordings, points at 15 min. intervals were taken to describe the data. Following the 30 mg injection of $PGF_{2\alpha}$, the ΔT_{u-a} dropped .4 C ($P<.01$) from approximately .54 C to .16 C at 45 min. postinjection (figure 15). A similar drop ($P\simeq.10$) in ΔT_{u-a} occurred following the 10 mg $PGF_{2\alpha}$ injection. However, the magnitude of the decline was only about .15 C which occurred 30 min. postinjection. The lower ΔT_{u-a} before injection of $PGF_{2\alpha}$ (10 mg) and

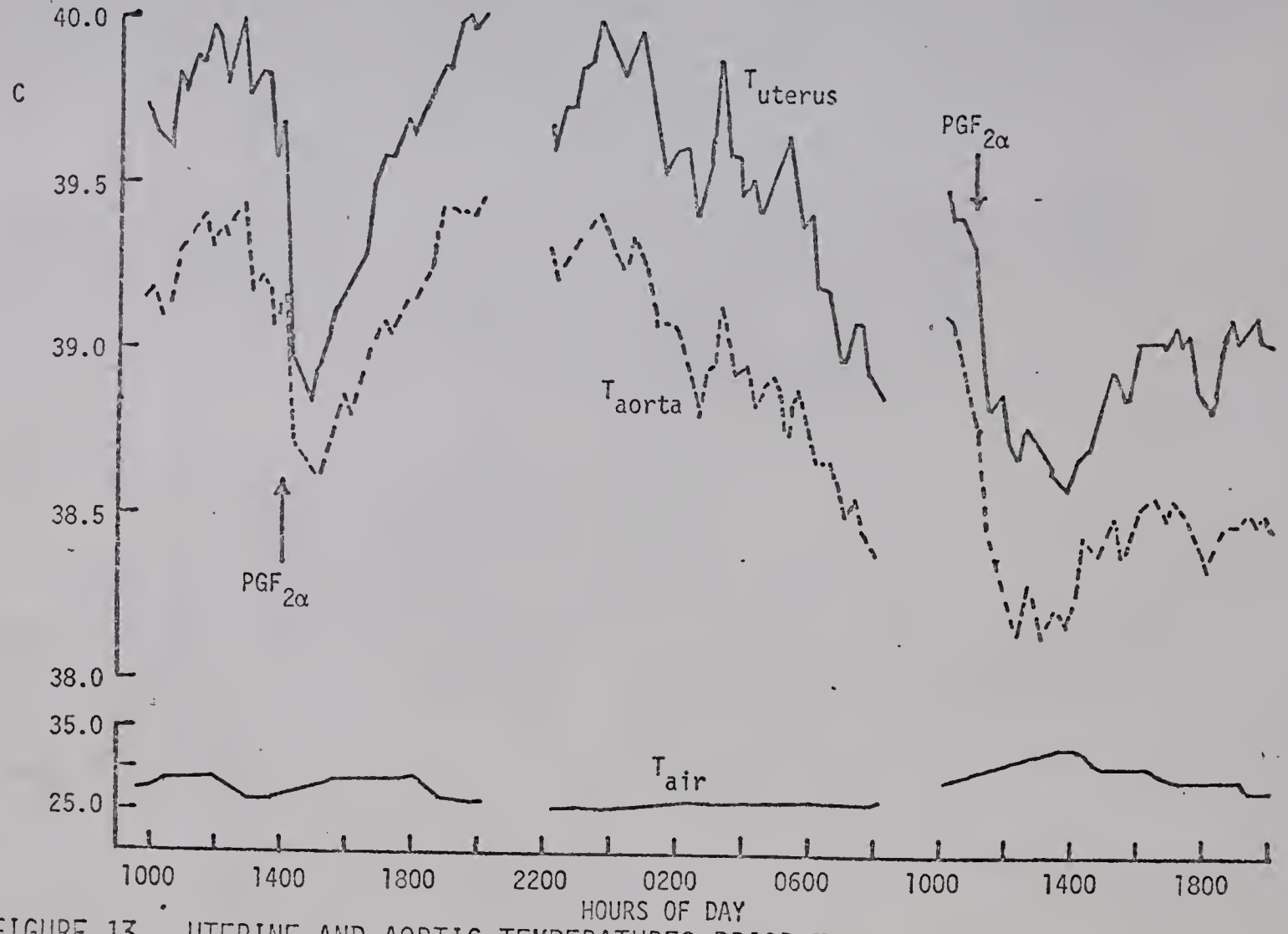

FIGURE 13. UTERINE AND AORTIC TEMPERATURES PRIOR TO AND FOLLOWING $PGF_{2\alpha}$ INJECTIONS IN G665.

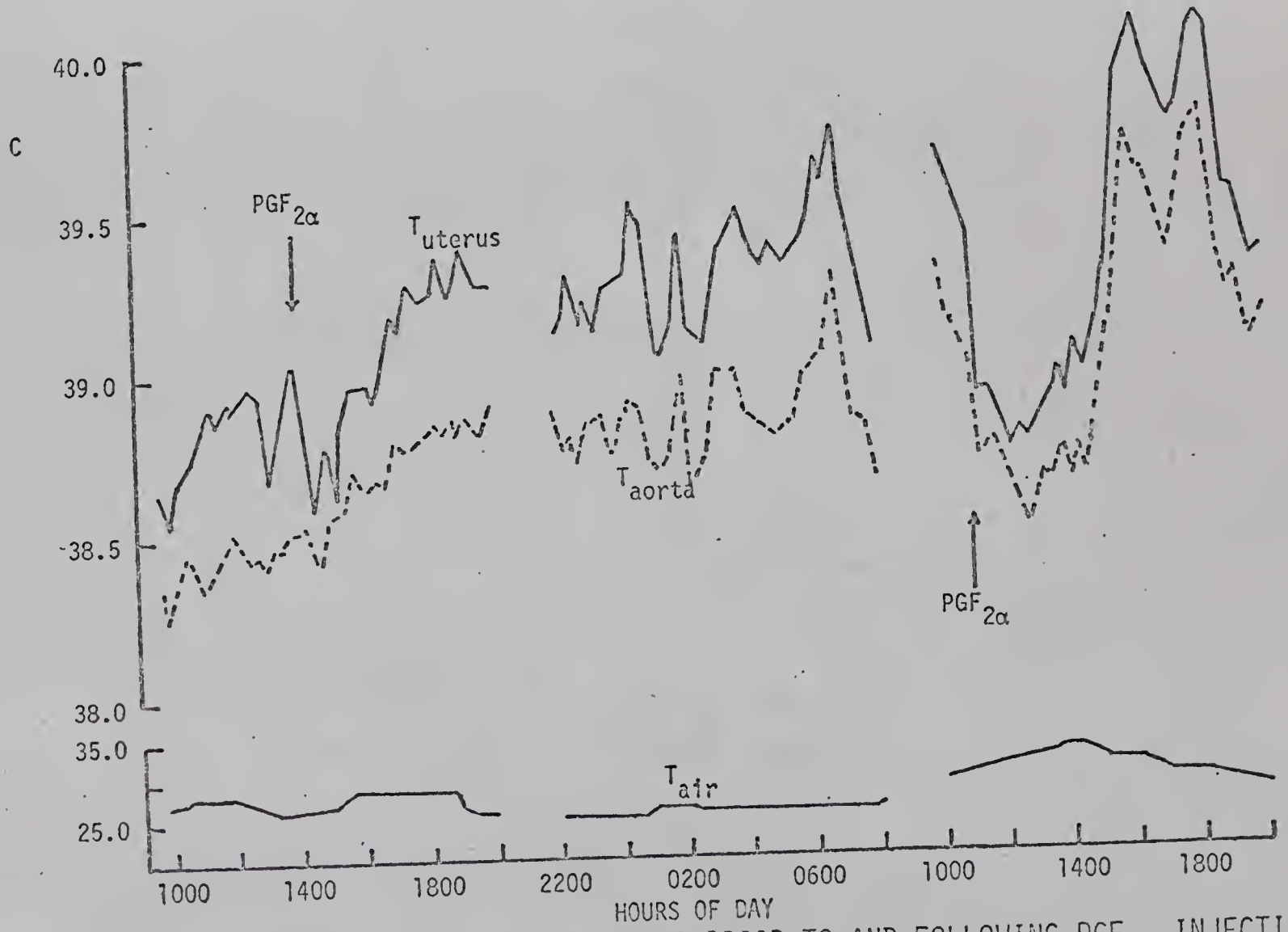

FIGURE 14. UTERINE AND AORTIC TEMPERATURES PRIOR TO AND FOLLOWING $PGF_{2\alpha}$ INJECTIONS IN JN15.

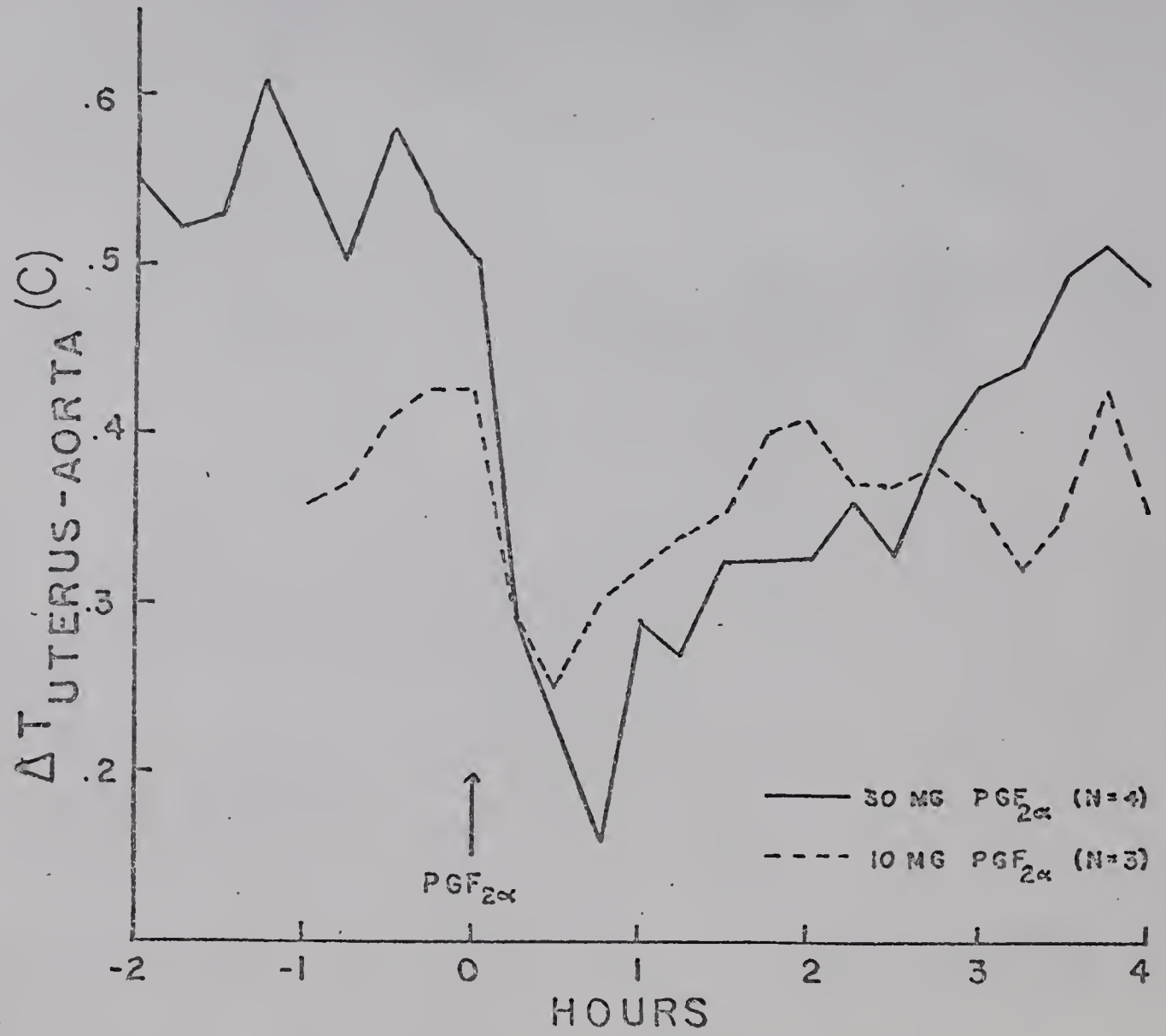

FIGURE 15. CHANGES IN ΔT_{u-a} FOLLOWING $PGF_{2\alpha}$ INJECTIONS.

smaller decline may be related both to time and hormonal status after first injection and also dose of $PGF_{2\alpha}$. These observations were not anticipated because various researchers (Bergstrom et al., 1968; Brody and Kodowitz, 1974; Clark et al., (1972) have reported a vasoconstrictor effect of $PGF_{2\alpha}$. A vasoconstrictor action would tend to decrease blood flow through the uterus and therefore elevate the ΔT_{u-a}. The marked drop in blood temperature might be attributed to an increased respiratory evaporative heat loss. Indeed, an increased respiratory rate was detected shortly after $PGF_{2\alpha}$ injection but not quantified. Sweating is negligible in cattle, so in order for heat to be eliminated by way of respiratory evaporative heat loss there has to be a tremendous increase in lung ventilation (Brody, 1945). Also, Lewis and Eyre (1972) reported increased respiratory volume following $PGF_{2\alpha}$ administration to calves. However, if aortic temperature did fall, a decrease in the ΔT_{u-a} would not occur unless there was selective $PGF_{2\alpha}$ action on the uterus to increase heat loss or decrease heat production.

If one uses the thermal balance equation, $Q=Fc\Delta T$, then theoretical heat production, Q, and uterine blood flow, F, can be calculated based on the ΔT_{u-a} changes.

Q = rate of uterine heat production (cal/gm tissue-min.)

F = rate of uterine blood flow (gm blood/gm tissue-min.), density of blood taken as 1 gm/ml

c = specific heat of blood (.87 cal/gm blood-C)

ΔT_{u-a} = temperature difference between the uterus and aortic blood (C); (adapted from Abrams et al., 1970b).

The major assumption is that all heat loss from uterine tissue is by way of the uterine veins. Therefore, in order to calculate Q, uterine blood flow during the luteal phase of the estrous cycle needs to be obtained. Assuming no species differences, then we can use for cattle the value of 119 ml blood/kg-min. for uterine blood flow in sheep (Huckabee et al., 1968).

Theoretically, at ΔT_{u-a} = .55 C just prior to $PGF_{2\alpha}$ injection in the present experiment:

$$Q = Fc\Delta T$$

Q = .119 gm blood/gm tissue-min. X .87 cal/gm blood-C X .55 C = .057 cal/gm-min.

By contrast, the uterine heat production rate, Q, calculated 45 min. post-$PGF_{2\alpha}$ injection when ΔT_{u-a} = .16 C was determined to be:

Q = .119 X .87 X .16 = .017 cal/gm-min.

This theoretical calculation would suggest a 3.4 fold decrease in uterine heat production in response to $PGF_{2\alpha}$.

Lowered ΔT_{u-a} in response to $PGF_{2\alpha}$ could also be explained by an increase in heat loss. One mechanism of heat loss would be an increase in uterine blood flow. Be rearranging the equation, the theoretical blood flow, before and after $PGF_{2\alpha}$, can be calculated based on oxygen consumption data for a 350 kg Jersey cow (oxygen consumption 3.26 ml/kg-min.; Brody, 1945). Assuming no differences between oxygen consumption (per kg) of various organs of the body (in sheep the oxygen consumption of the total body as well as the uterus is approximately 5 ml/kg-min.), oxygen consumption of uterine tissue of 3.26 ml/kg-min.

multiplied by a calorific value of 4.8 cal/ml oxygen (based on an assumed R.Q. of .8; Brody, 1945) would give a calculated heat production of 3.26 ml/kg-min. X 4.8 cal/ml = .0156 cal/gm-min.

Rearranging the original Equation:

$$F = \frac{Q}{c\Delta T}$$

then at ΔT_{u-a} = .55 C (pre-$PGF_{2\alpha}$ injection):

$$F = \frac{.0156 \text{ cal/gm-min.}}{.87 \text{ cal/gm blood-C X } .55 \text{ C}} =$$

.0326 gm/gm-min.

or

32.6 ml blood/kg-min.

and at ΔT_{u-a} = .16 C (post-$PGF_{2\alpha}$ injection):

$$F = \frac{.0156}{.87 \text{ X } .16} =$$

.122 gm/gm-min.

or

112 ml blood/kg-min.

The 3.4 fold increase in theoretical uterine blood flow calculated above would be comparable to changes reported by various researchers (Huckabee et al., 1970; Abrams et al., 1970a) following estrogen injections. However, the time course of maximum $PGF_{2\alpha}$ response (45 min.) was of shorter duration and had a more rapid onset than an estrogen induced decrease in ΔT_{u-a} (Section III, Experiment 1). These observations indicate the need for more definitive experiments in the bovine to pinpoint the cause of the lowered ΔT_{u-a} in response to $PGF_{2\alpha}$. A basic question is whether there is an increase in uterine blood flow or a

decrease in heat production.

Figures 13, 14, 16 and 17 show individual cow aortic and uterine temperature changes pre- and post $PGF_{2\alpha}$ injection (figures 13 and 14) and at the time of the LH peak (figures 16 and 17). Both cows appeared to exhibit circadian changes in aortic and uterine temperatures. The range of aortic temperatures (37.9 to 41.0 C) within the two cows is comparable to body temperatures reported by Bligh and Harthoorn (1965) in African cattle. They reported that maximum and minimum body temperatures were closely associated with sunset and sunrise, respectively. In the latter study thermistors were implanted 8 cm into the dorsal caudal neck region to record deep body temperature. Aortic temperature patterns in our study showed that maximum daily deep body temperature occurred close to midnight, whereas minimal body temperature occurred between 0800 and 1200 hr. Although cows were turned out to exercise at 0800 and 2000 hr., their aortic temperatures returned to pre-turnout baselines within 2 hr. after their return to the barn. Also, there appears to be a 4 to 6 hr. lag behind barn air temperature in maximum and minimum body temperature.

The uterine and aortic temperatures were highly correlated (Appendix, table 13) but no correlation between ΔT_{u-a} and either uterine or aortic temperature was detected. This might suggest that there was no change in uterine blood flow and uterine heat production. However, these correlations were based on data throughout the entire experiment and any possible increases in ΔT_{u-a} at higher body temperatures (figures 16 and 17) may have been undetected statistically. Visual appraisal of figures 16 and 17 do show a widening of the ΔT_{u-a} at maximum daily

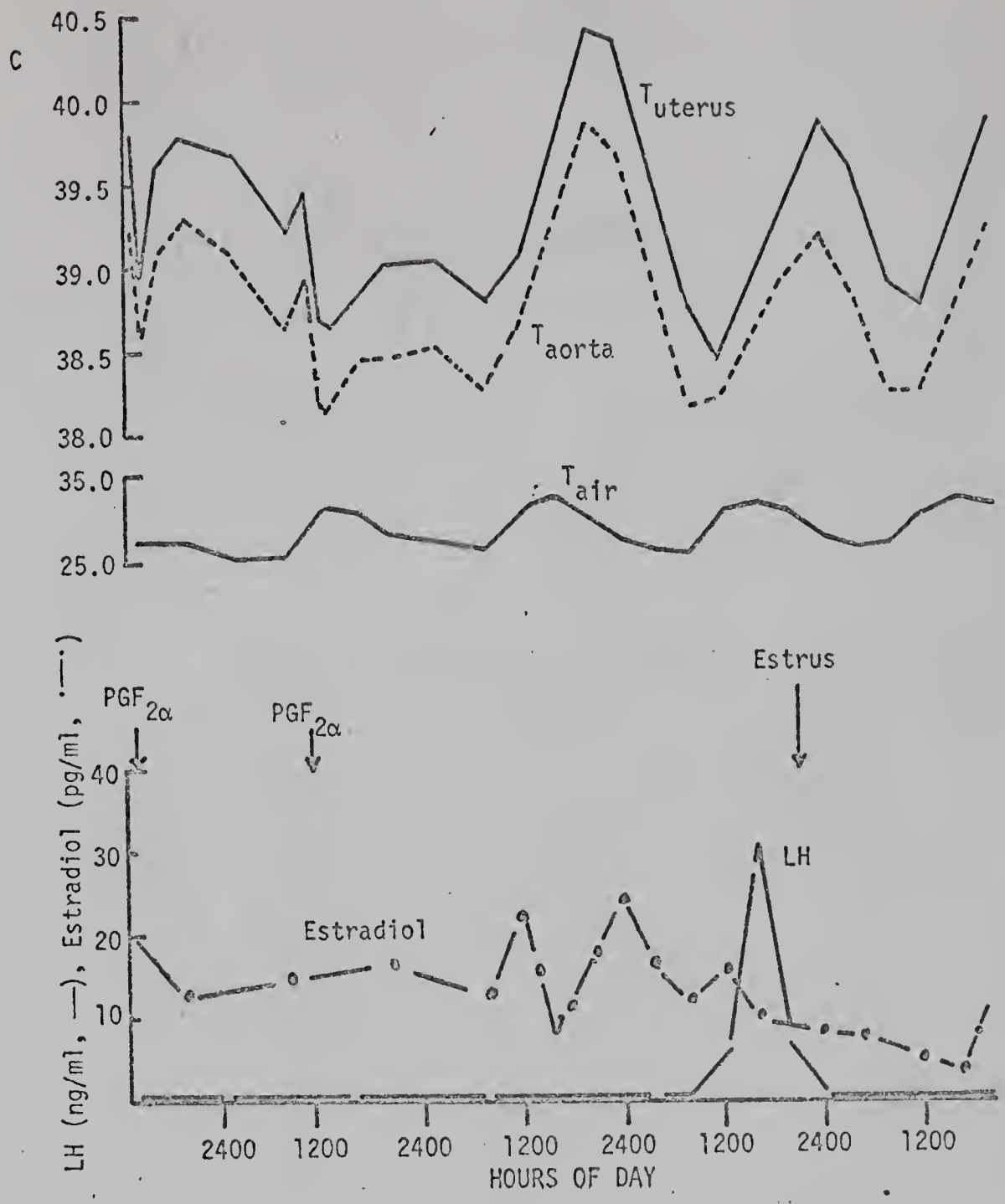

FIGURE 16. UTERINE AND AORTIC TEMPERATURES, LH AND ESTRADIOL IN G665 AND AIR TEMPERATURES.

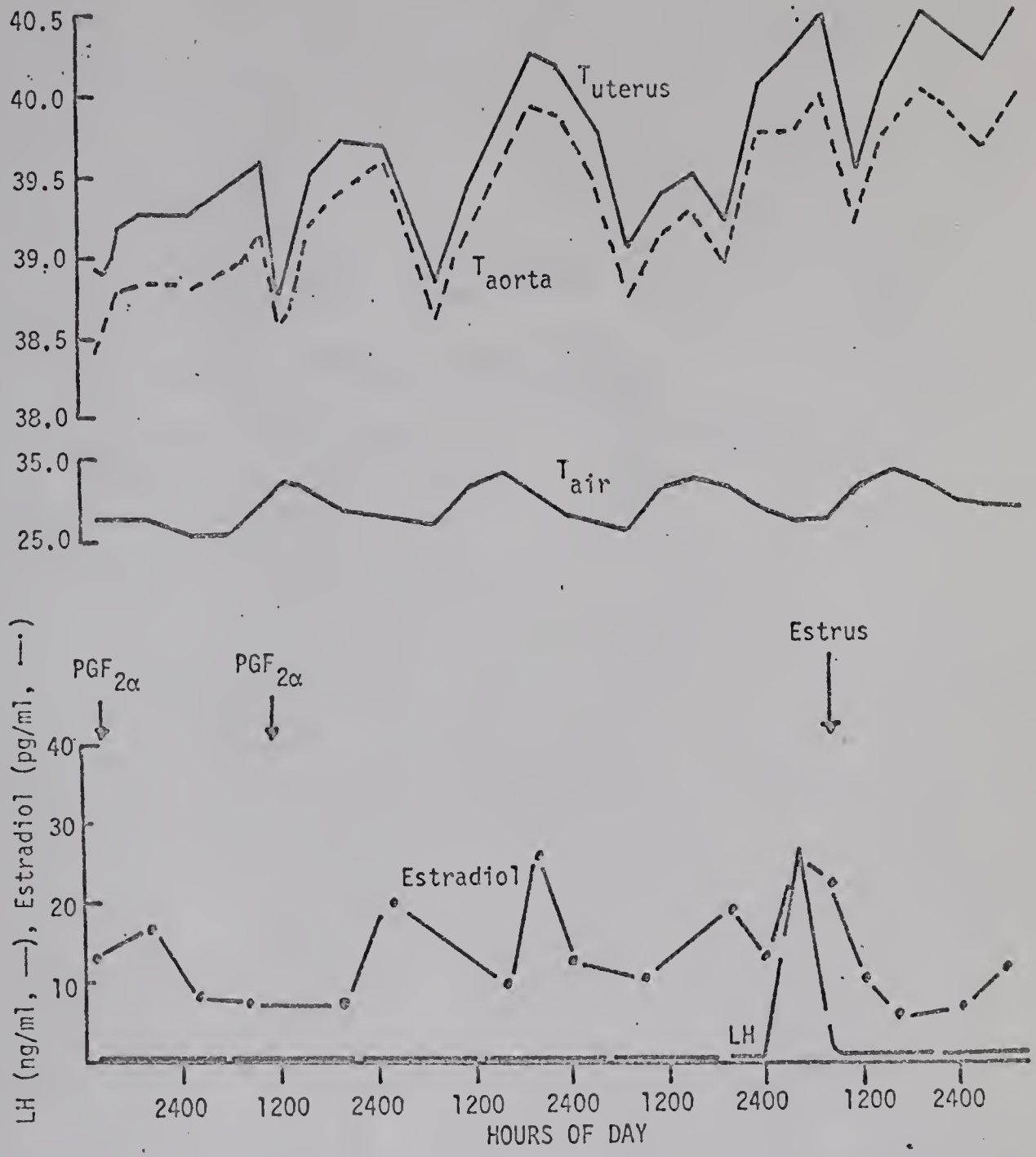

FIGURE 17. UTERINE AND AORTIC TEMPERATURES, LH AND ESTRADIOL IN JN15 AND AIR TEMPERATURES.

uterine and aortic temperatures.

Figure 18 shows the significant ($P<.01$) curvilinear (2nd order) time trends for uterine and aortic temperatures when data were pooled across days for each time of blood sampling. The data representing each individual sampling point is the average of individual 15 min. points $\pm$ 2 or 3 hr. from time of the blood sample. Also only temperatures preceding turn out of cows (0800 and 2000 hr.) were used in obtaining an average for the 0800 and 2000 hr. blood sampling times. The air temperature plot is comprised of average values across the individual days. Uterine temperature and aortic temperature were not influenced by barn air temperature ($P>.10$; Appendix, table 13). The uterine temperature trend throughout the day was best described by $\hat{Y}_{(\text{uterine temperature, C})} = 40.04 - .143X + .007X^2$ ($P<.01$) where X = hr., whereas aortic temperature was best characterized by $\hat{Y}_{(\text{aortic temperature, C})} = 39.50 - .125X + .006X^2$ ($P<.01$; Appendix, table 14). The body temperature lag of about 6 hr. behind air temperature (1600 hr.-peak air temperature compared to 2400 hr. body temperature peak) is best seen in figure 18.

Possible explanations for the time delay could be:

1) Thermal inertia of the cow. For example, ambient temperature will increase more rapidly than body temperature because of the mass and heat capacity of the large mammal.

2) Inherent circadian rhythm of body temperature which may be independent of environmental temperature and cued to external events such as light-dark cycles, feeding regimen, presence of barn personnel and other factors which were uncontrolled in this experiment.

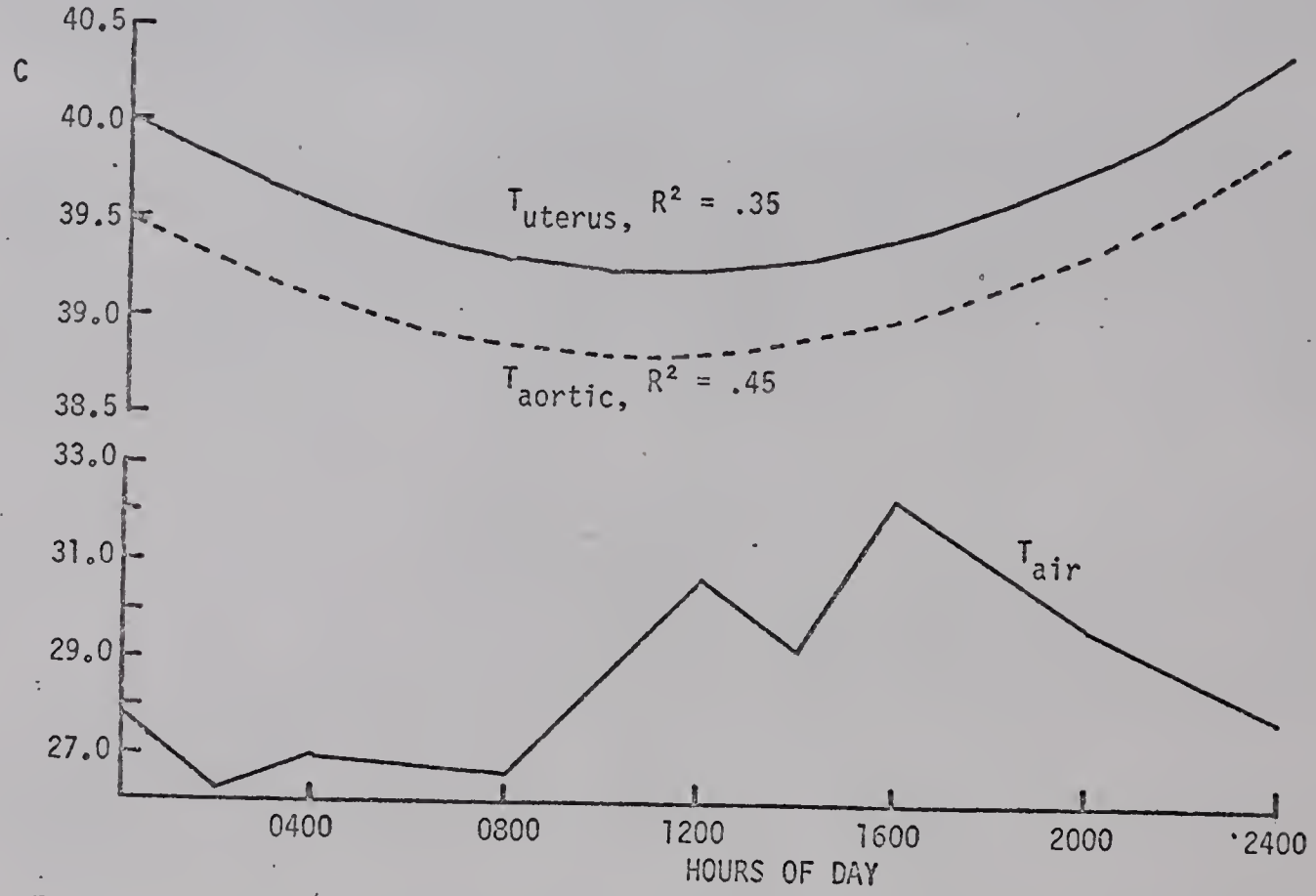

FIGURE 18. CIRCADIAN UTERINE, AORTIC AND AIR TEMPERATURE CHANGES.

Correlated responses between concurrent temperature measurements (uterine temperature, aortic temperature and air temperature) are low due to the time delay phenomena.

Of interest in this experiment is the observation that uterine temperatures during the day reached 40 C for periods of up to 6 hr. as ambient temperature fluctuated near 30 C (figures 16 and 17). Temperatures of this magnitude (40 C) are damaging to embryo development at the 1 to 4 cell stages (Alliston et al., 1965). In JN15 (figure 17) this increased uterine temperature was at the time when artificial insemination would normally have been performed.

We failed to detect an association between concurrent measurements of ΔT_{u-a} with estradiol or LH (Table 13). Based upon Experiment 1 there was a 2.5 hr. delay between injection of a pharmacological dose of estradiol and the minimum ΔT_{u-a}. From $PGF_{2\alpha}$ injection through the LH surge (figure 19) estradiol concentrations fluctuated considerably as did the ΔT_{u-a} (figures 16 and 17). Not until the massive LH discharge was there an appreciable rise in ΔT_{u-a} at a time when estradiol was decreased (figure 19).

Findings of this experiment indicate that uterine and aortic temperatures followed a daily circadian rhythm, and, because of a time lag in these temperatures behind air temperatures, correlations between body temperatures and ambient temperature were negligible. Failure to detect an association between ΔT_{u-a} and hormonal measurements may be due to the time lag, also, The mild heat stress (which by definition occurs in cattle anytime ambient temperatures exceed 30 C), to which these cows were subjected to, may have contributed to the high uterine and aortic blood temperatures. Uterine temperatures periodically

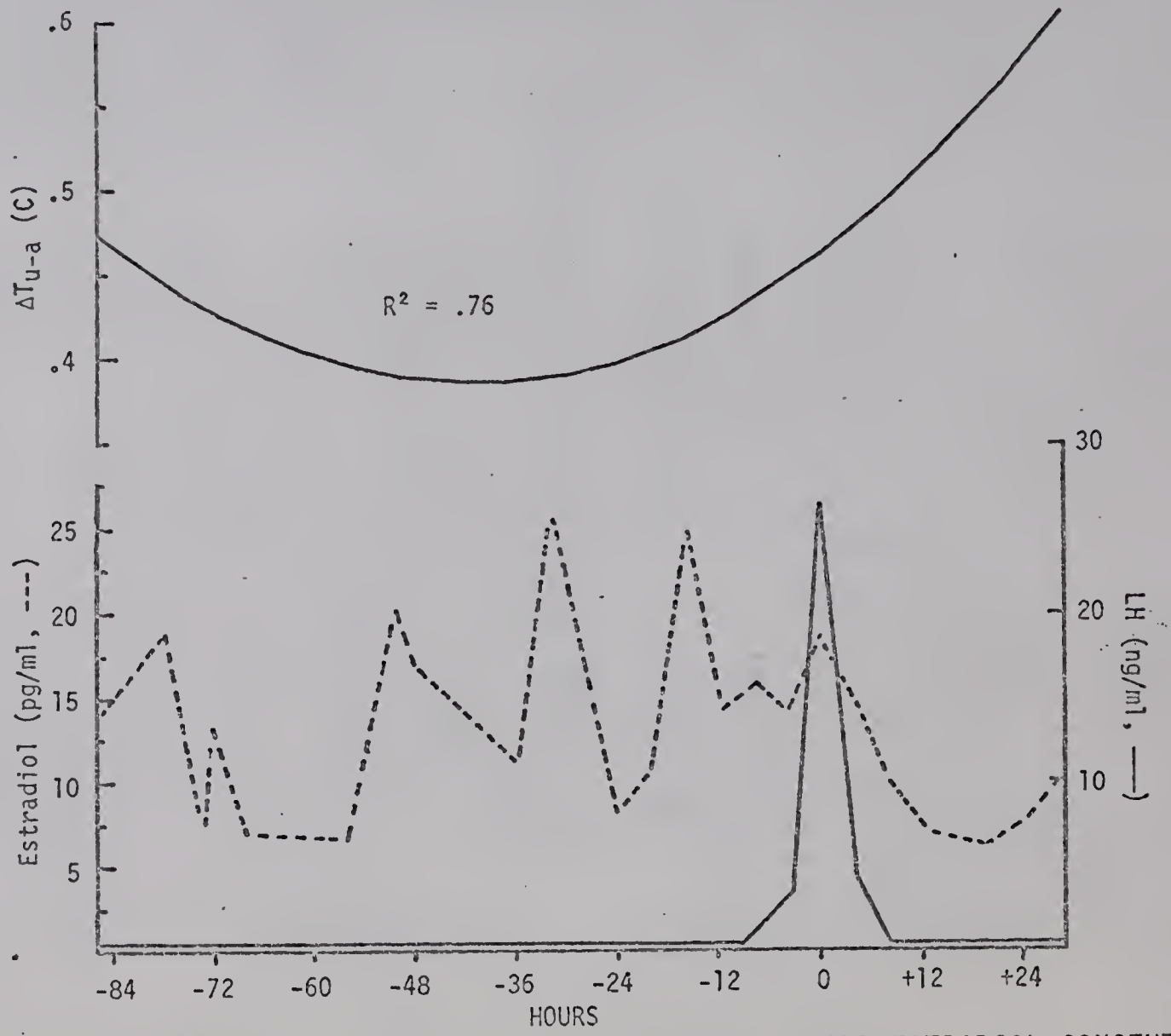

FIGURE 19. CHANGES IN ΔT_{u-a} ASSOCIATED WITH ENDOGENOUS LH AND ESTRADIOL CONCENTRATIONS.

exceeded 40 C (Appendix, table 15). Since survival rate of fertilized ova exposed to 40 C for 3 hr. is seriously decreased, these observations may be of some practical significance in improving reproductive performance in a hot climate.

SECTION IV

SUMMARY AND CONCLUSIONS

Ten normally cycling Holstein heifers at the USDA, Agricultural Research Center, Beltsville, Maryland, were assigned to one of two environmental treatment groups (21.3 C, 59% RH or 32.0 C, 67% RH). $PGF_{2\alpha}$-Tham Salt ($PGF_{2\alpha}$) was used to cause corpus luteum regression and synchronize estrus. Blood samples were collected prior to $PGF_{2\alpha}$ injection and at 6 or 4 hr. intervals following injection through ovulation. Plasma samples were analysed to determine concentrations of progestins, estradiol, estrone, LH, prolactin, corticoids, total protein concentration, osmolality, cortisol binding capacity and cortisol association constants. In the second phase of this first experiment adrenal responsiveness to ACTH (200 IU) was tested by quantification of corticoid concentrations in plasma prior to and up to 12 hr. following injection of ACTH. Least-squares analyses were conducted to characterize treatment, animal and within-animal time trends in plasma progestins, estradiol, estrone, LH, prolactin and corticoids. Other response variables were analyzed by analysis of variance.

Environmental treatment of 32.0 C evoked a 1.49 C increase in rectal temperature and a 3.59 C increase in skin temperatures. Time durations between $PGF_{2\alpha}$ injection to LH peak and ovulation were not different ($P>.10$) between treatments. Length of estrus was shorter

(P<.10) for heat stressed heifers (21 compared to 16 hr.). Two of four heifers inseminated at 21.3 C were pregnant at 40 days compared to none of five at 32.0 C. Thus, the environmental thermal stress did affect body temperature, duration of estrus and overall fertility.

No differences (P>.10) in plasma samples, prior to injection of $PGF_{2\alpha}$, were detected due to main effects of temperature. Hormonal responses post-injection between treatments were detected. Average progestin concentration between treatments was not different (P>.10), however, 5th order regression curves were not parallel (P<.01) indicating different time responses between treatments. Progestin concentrations declined in a similar manner in both groups immediately following $PGF_{2\alpha}$ injection. Heifers in the 21.3 C group had an LH surge about 24 hr. later than heifers in the 32.0 C group. This 24 hr. time lag accounted for the difference in time responses when data were synchronized to time of LH peak for analysis. Mean estradiol concentration was significantly (P<.10) lower in heat stressed heifers. The lower plasma estradiol may have contributed to the shorter periods of estrus in the 32.0 C heifers. However, these lower concentrations of estradiol were adequate enough to elicit estrous behavior and trigger a preovulatory surge of LH to cause ovulation.

Estrone showed no apparent association with either onset of estrus or peak of LH when the data were synchronized to time of the LH peak. There was a significant elevation (P<.05) of estrone due to heat stress, but there was no evidence that estrone time trends following $PGF_{2\alpha}$ were not parallel (P>.10). In both treatments, estrone followed a similar decline postinjection. No differences (P>.10) were found in mean

LH concentrations between heifers at 21.3 C or 32.0 C. Plasma pre-ovulatory peak of LH concentrations were 32.2 and 33.2 ng/ml for 21.3 C and 32.0 C treated heifers, respectively. All animals had a pre-ovulatory LH surge indicating that hyperthermia did not prevent the triggering mechanism for LH release.

There was no change in prolactin associated with estrus or peak of LH, therefore, prolactin was analyzed relative to time of $PGF_{2\alpha}$ injection. Mean prolactin concentrations were not different between treatments ($P>.10$), but 4th order response time curves were not parallel ($P<.005$). Heifers in the 21.3 C chamber had a decline in plasma prolactin associated with sequential blood sampling, whereas prolactin increased in 32.0 C treated heifers during the early blood sampling period. The decline of prolactin in cooler heifers may be related to a lowering of stress-induced prolactin secretion associated with initial sampling. Apparently heifers in the 32.0 C chamber could not adjust to sampling as quickly since prolactin remained elevated until 24 hr. after $PGF_{2\alpha}$ (42 hr. after the initial sample). The summer seasonal increase in plasma prolactin, reported by various researchers, may be more related to photoperiod. There was no difference ($P>.10$) between treatments in plasma corticoid concentrations. The coefficient of variation for plasma corticoid was 65% after accounting for variability due to treatment, heifers within treatment and time trends up to the 5th order.

In an attempt to determine if plasma dilution may have occurred, total protein concentration and osmolality were measured. There was no difference ($P>.10$) in total protein concentration or osmolality between treatment groups. However, no measurement of total plasma volume was

made. Cortisol binding capacity of CBG and its association constant (K_a) were determined. The affinity (K_a) of cortisol for CBG was not different between treatments ($P<.10$); however, the binding capacity of CBG for cortisol was reduced ($P<.05$) in the 32.0 C heifers. This observation suggested that, under experimental conditions (4 C) for determining cortisol binding capacity, hyperthermic heifers had a decreased plasma concentration of CBG.

Results of this first experiment show only subtle thermal effects on plasma estradiol and estrone concentrations, and no effects on plasma LH, progestins, corticoids and prolactin. Apart from possible hormonal involvement with duration of estrus, heat stress does not appear to affect drastically the peripheral plasma hormonal milieu associated with corpus luteum regression, follicle growth and ovulation.

At 8 days following ovulation in the last heifer of the first experiment, the 10 heifers were injected with 200 IU ACTH (IV). The 32.0 C heifers responded with significantly lower ($P<.10$) corticoid concentrations. The 6th order regression response curves were not parallel ($P<.01$) suggesting that the hot group response was earlier to reach a peak (75 min. compared to 105 min.), had a lower magnitude (73.5 compared to 100.2 ng/ml corticoids) and was of shorter duration (4 hr. compared to 5 hr.). The significance of a possible lowered adrenal function in hot environments may be related to state of lowered heat production. Since corticoids are known to be calorigenic, a lowered adrenal responsiveness in hyperthermic heifers might be physiologically advantageous. However, a possibly lowered adrenal responsiveness at 32 C was detected only after stimulation of

the adrenal with ACTH.

Because the first experiment did not specifically consider possible environmental and hormonal effects on uterine temperature, it was necessary to document possible hormonal and environmental effects on uterine thermal changes. Estrogen induced thermal changes were documented and an attempt was made to characterize uterine thermal changes during the period of luteal regression, follicle growth and ovulation under conditions of mild heat stress.

In experiment two, thermocouples were placed into the uterine serosa and aortic blood vessel of four dairy heifers. Injection of 3 mg estradiol-17β caused a .25 C decrease ($P<.01$) in the difference between uterine and aortic temperature (ΔT_{u-a}) by 2.5 hr. postinjection. In contrast there was no significant change ($P>.10$) in ΔT_{u-a} after injection of saline. An augmented rate of heat loss resulting from a marked estradiol-17β induced elevation in uterine blood flow may account for the decreased difference between uterine and aortic temperature after estradiol-17β injection.

The final experiment was an attempt to document and evaluate changes in uterine temperature during the period of luteal regression (decreasing progesterone), follicle growth (increasing estradiol) and ovulation induced by $PGF_{2\alpha}$ under conditions of a mild heat stress. Thermocouples were placed into the uterine serosa and aortic blood vessel of four dairy cattle. Blood samples were collected at 6 to 4 hr. intervals following $PGF_{2\alpha}$ injection in order to monitor endogenous peripheral plasma concentrations of estradiol and LH. $PGF_{2\alpha}$ caused an immediate drop in uterine and aortic temperatures, and a decrease in the ΔT_{u-a} of almost

.4 C at 45 min. postinjection. This effect of $PGF_{2\alpha}$ on the uterus may be due to a selective vasodilatory response at the uterus. The $PGF_{2\alpha}$ induced transient decline in ΔT_{u-a} in the bovine needs further clarification.

Two cows, in which thermocouples remained operational for the duration of the study, had monophasic daily uterine and aortic temperature rhythms. However, both temperatures lagged about 6 hr. behind air temperature changes. Thermal inertia or inherent circadian rhythms of the body may be involved in the time delay. Uterine temperatures reached 40 C for periods of up to 6 hr. During this time ambient temperature fluctuated around 30 C (mild heat stress). Failure to detect an overall association between concurrent ΔT_{u-a} and hormonal measurements might have been due to a time lag association and estradiol variability following $PGF_{2\alpha}$ injection. Not until the preovulatory surge of LH was there an appreciable rise in ΔT_{u-a} ($P<.01$), and this occurred at a time when plasma estradiol was decreasing. The mild environmental heat stress may have contributed to the high uterine and aortic blood temperatures. Since survival rate of fertilized ova exposed to 40 C for 3 hr. is seriously decreased, interrelationships in the last experiment between ambient, uterine, aortic temperatures and hormonal status may have major practical implications.

In conclusion, under experimental conditions of the above study, heat stress caused only subtle effects on peripheral plasma hormonal concentrations during the preovulatory period. Adrenal responsiveness to ACTH appears to be altered under conditions of thermal stress. Uterine temperatures are sensitive to estradiol, $PGF_{2\alpha}$ and ambient temperatures.

APPENDIX

Table 4. Overall least squares analyses of variance for hormones in heifers at 21.3 C and 32.0 C[a].

SOURCE	d.f.	PROGESTINS[b] MS	LH[b] MS	ESTRADIOL[b] MS	ESTRONE[c] MS	PROLACTIN[c] MS	CORTICOIDS[b] MS
Treatment[d]	1	4.6	3.2	22.5[e]	3.9*	34.3	.7
Heifers/21.3 C	4	4.6*	3.9*	1.5	.5	217.0**	58.4**
Heifers/32.0 C	4	1.8	9.1**	10.6**	.7	610.8**	35.5**
TIME							
Linear	1	1.5	143.4	236.1	21.1	6.9	25.5
Quadratic	1	7.8	163.2	286.2	28.0	6.3	36.6
Cubic	1	9.0	94.6	191.8	25.1	5.2	28.6
Quartic	1	2.0	2.6	2.6	21.8	5.1	1.5
Quintic	1	4.3**	155.8[e]	215.2**	19.1**	6.4	86.7[e]
Remainder	276	.52	46.52	6.16	1.41	36.26	26.24

[a] ($\bar{X}\pm SD$; 21.3 C - n=152, 32.0 C - n=125), Progestins - .63±1.03; .65±1.1, LH - 3.08±6.37; 3.35±7.42, Estradiol - 3.45±3.09; 2.96±2.50, Estrone - 1.55±1.27; 1.85±1.56, Prolactin - 14.51±7.64; 14.78±6.0, Corticoids - 8.01±6.04; 7.76±4.09.

[b] Synchronized to the time of the LH peak

[c] Synchronized to the time of the $PGF_{2\alpha}$ injection

[d] Treatments tested by pooling heifer/temperature sums of squares to get a pooled mean square

**(P<.01), *(P<.05), [e](P<.10)

Table 5. Plasma progestins (ng/ml) following $PGF_{2\alpha}$ injection.

Hours from $PGF_{2\alpha}$	21.3 C					32.0 C				
	ANIMAL NO.									
	63	68	87	89	94	62	64	86	88	92
-18	1.13	2.73	5.07	3.33	4.33	4.00	5.87	4.27	3.60	1.00
-12	.90	3.47	2.47	3.80	3.53	3.35	4.73	1.70	4.67	1.85
0	1.90	3.55	4.53	4.13	3.33	3.10	3.20	2.90	2.20	1.05
6	.90	1.75	3.55	2.55	2.55	2.95	3.60	2.65	1.37	1.38
12	.60	.75	.45	.77	.50	1.33	.80	.70	.60	.33
18	.35	.90	.65	.55	.60	.50	.70	.65	.73	.30
24	.20	.28	.81	.63	.61	.33	.60	.32	.35	.32
30	.54	.57	.95	.35	.27	.23	.90	.57	.42	.13
36	.20	.17	.77	.18	.15	.28	.33	.25	.26	.16
42	.20	.24	.55	.24	.23	.22	.25	.40	.18	.13
48	.17	.13	.47	.28	.16	.20	.23	.39	.13	.12[a]
52	.25	.14	.41	.18	.11	.22	.16	.28	.19[a]	.17
56	.30	.16	.53	.18	.11	.22	.18	.27	.18	.11
60	.22	.14	.16	.11	.12	.14	.26	.17	.10	.05
64	.30	.06	.51	.22	.05	.15	.14	.18	.15	.08
68	.30	.12[a]	.62	.17	.14[a]	.37	.15	.15	.12	.07
72	.29	.07	.43	.24	.04	.27	.23	.34	.16	.10
76	.32	.17	.29	.17	.18	.17[a]	.17	.25	.16	.13
80	.24	.12	.22	.11	.08	.15	.24	.09	.15	-
84	.35	.25	.20	.17	.13	.09	.28[a]	.28	.16	.11
88	.21	.07	.39	.44	.35	.30	.16	.17	-	-
92	.53	.32	.47	.30	.29	.40	.32	.48	-	-
96	1.09	.38	.77	.26	.26	.26	.28	.48	.42	.16
100	.64	-	.09	.10[a]	-	.04	.04	.10	-	-
104	.17	-	.09	.07	-	-	.05	.17[a]	-	-
108	.15	-	.17[a]	.15	.05	.17	.02	.15	.08	.08
112	.18	-	.05	.05	-	-	-	.14	-	-
116	.15	-	.04	.03	-	-	-	.09	-	-
120	.15	.14	.07	.07	.14	.04	.13	.08	.17	.40
124	.24	-	.06	.09	-	-	-	.10	-	-
128	.18[a]	-	.05	-	-	-	-	.08	-	-
132	.21	.10	.04	.24	.16	.12	.14	.09	.27	.36
136	.17	-	.07	-	-	-	-	-	-	-
140	.53	-	-	-	-	-	-	-	-	-
144	.10	.24	.05	.11	.35	.13	.15	.05	.34	.60

[a]Time of LH peak

Table 6. Plasma estradiol (pg/ml) following $PGF_{2\alpha}$ injection.

Hours from	21.3 C					32.0 C				
$PGF_{2\alpha}$	ANIMAL NO. 63	68	87	89	94	62	64	86	88	92
-18	3.99	3.34	2.77	5.18	2.55	2.56	2.72	3.09	.53	3.36
-12	6.76	1.46	1.65	2.78	1.74	1.80	2.04	1.70	1.37	5.00
0	5.93	1.26	2.03	1.32	2.48	1.70	2.42	1.40	2.14	2.97
6	1.55	2.39	1.43	3.39	1.84	2.13	2.16	2.95	.89	5.38
12	.68	1.27	.72	1.18	1.46	1.15	.75	.93	1.79	1.44
18	.50	.72	.89	.96	.98	1.20	.89	.87	2.00	2.38
24	1.19	1.68	1.77	1.14	1.89	2.21	1.41	1.56	3.51	2.86
30	1.53	2.84	1.10	1.27	1.44	2.03	1.74	2.68	2.05	2.22
36	.50	1.72	.88	1.69	2.27	1.29	1.73	1.67	2.59	2.40
42	1.32	1.39	1.55	1.25	1.03	1.23	1.41	1.68	5.75	3.54[a]
48	1.65	1.09	1.20	1.13	1.38	1.63	2.66	1.50	3.01[a]	2.59
52	1.16	1.69	.97	4.19	8.59	1.53	1.27	1.18	3.29	6.30
56	3.85	6.02	3.01	3.78	7.21	5.91	6.06	7.29	8.38	2.24
60	2.17	12.51	1.96	5.16	9.64	4.76	4.30	2.87	2.85	1.46
64	1.00	10.05[a]	1.00	7.24	16.52[a]	5.07	3.34	1.00	1.00	1.00
68	1.00	13.42	1.00	7.02	10.42	-	5.12	1.00	1.00	1.00
72	2.54	6.62	6.71	4.08	4.38	4.95[a]	7.74	5.13	1.85	.57
76	3.31	1.57	1.92	4.86	3.60	12.17	8.32	4.87	1.75	1.23
80	2.83	3.01	4.90	4.78	2.68	8.46	7.76[a]	2.95	1.38	-
84	4.78	2.29	4.30	4.57	1.53	3.66	9.68	6.37	.54	1.51
88	4.05	2.95	8.01	7.07	2.21	5.39	5.71	6.84	-	-
92	5.43	1.91	8.21	6.83	2.07	1.78	3.27	7.81	-	-
96	4.83	2.17	6.60	7.28[a]	1.02	2.25	2.91	7.13	1.61	1.61
100	4.27	-	5.25	9.68	-	1.38	1.03	14.67[a]	-	-
104	3.09	-	4.59[a]	5.08	-	-	1.77	7.86	-	-
108	3.19	-	9.95	.50	.50	.69	.58	4.69	.50	8.24
112	9.49	-	3.98	.50	-	-	-	.50	-	-
116	12.47	-	1.18	.50	-	-	-	.72	-	-
120	9.77	.50	5.41	4.47	3.41	.50	1.79	1.17	.50	2.38
124	11.01[a]	-	6.34	.98	-	-	-	5.21	-	-
128	8.03	-	.69	-	-	-	-	1.84	-	-
132	2.60	.66	.50	-	1.04	.82	.99	1.34	1.25	1.15
136	1.38	-	1.06	-	-	-	-	-	-	-
140	1.35	-	-	-	-	-	-	-	-	-
144	.74	1.77	.96	1.12	2.06	2.27	4.46	1.67	2.04	1.79

[a]Time of LH peak

Table 7. Plasma estrone (pg/ml) following $PGF_{2\alpha}$ injection.

Hours from $PGF_{2\alpha}$	21.3 C					32.0 C				
	ANIMAL NO.									
	63	68	87	89	94	62	64	86	88	92
-18	2.95	2.18	3.81	3.87	3.67	.64	1.07	2.52	1.00	4.51
-12	.98	.86	4.76	1.83	.70	6.09	.50	4.13	1.87	5.83
0	5.41	4.08	3.40	5.91	1.26	5.43	3.82	1.00	2.98	7.41
6	5.35	3.76	4.64	7.63	7.18	2.75	4.59	2.20	1.00	6.20
12	1.21	1.67	.75	1.49	1.92	2.08	1.15	3.02	3.93	.96
18	1.31	1.46	.76	2.37	2.50	1.43	1.79	1.50	1.73	2.47
24	2.74	1.41	1.86	1.00	2.84	5.13	9.99	2.27	2.55	2.14
30	1.87	2.50	1.06	1.17	1.73	6.62	1.72	2.85	1.17	1.17
36	.86	2.11	1.08	1.13	.79	1.76	1.62	1.50	2.34	1.02
42	.75	1.00	2.39	2.78	1.94	1.02	.75	3.45	3.93	2.38
48	1.25	2.45	2.24	1.29	2.30	1.75	1.70	2.28	3.17	3.19[a]
52	1.18	1.77	1.33	.50	1.00	1.18	2.27	1.12	1.71[a]	.50
56	.50	1.00	.50	.50	1.00	1.00	1.00	1.00	1.21	.50
60	.60	1.00	1.16	1.30	1.27	1.00	1.00	.73	.50	.50
64	2.09	1.32	1.00	1.46	1.58	2.70	1.18	1.00	1.77	1.00
68	1.14	1.65[a]	1.30	1.24	1.27[a]	-	1.48	1.00	1.00	1.75
72	.50	1.31	1.00	1.00	.50	2.48	1.30	1.00	1.71	.50
76	.50	.92	1.00	1.00	.50	2.11[a]	2.41	1.00	.50	.50
80	.50	.50	1.40	1.00	.93	1.14	1.00	1.00	.50	-
84	1.45	1.04	4.31	2.62	.81	.87	4.09[a]	1.58	.81	2.01
88	.50	.50	3.40	2.09	.73	1.21	1.04	1.61	-	-
92	.59	.75	1.00	1.00	1.27	.93	1.23	2.94	-	-
96	.50	1.75	1.73	1.00	.50	1.76	2.58	2.39	1.31	.50
100	2.01	-	1.00	1.75[a]	-	.50	.50	1.00	-	-
104	1.78	-	1.50	1.24	-	-	2.74	3.39	-	-
108	1.00	-	1.13[a]	.50	.50	1.27	.50	.50	.50	.75
112	1.00	-	.50	.50	-	-	-	.94	-	-
116	1.11	-	.82	.85	-	-	-	.50	-	-
120	1.00	1.63	.75	.82	1.29	.50	.50	.71	.50	.86
124	1.00	-	.75	.50	-	-	-	1.05[a]	-	-
128	1.00[a]	-	.82	-	-	-	-	.50	-	-
132	.72	.50	.50	-	.64	.50	.50	1.58	1.33	.50
136	.84	-	.50	-	-	-	-	-	-	-
140	.50	-	-	-	-	-	-	-	-	-
144	.50	1.76	.83	.50	1.59	.50	1.17	2.53	1.96	1.17

[a]Time of LH peak

Table 8. Plasma LH (ng/ml) following $PGF_{2\alpha}$ injection.

Hours from $PGF_{2\alpha}$	21.3 C					32.0 C				
	ANIMAL NO.									
	63	68	87	89	94	62	64	86	88	92
-18	2.79	.20	.20	.55	.43	.65	.50	.20	.20	2.35
-12	.60	.20	.20	.20	.87	.20	.70	2.08	2.59	.91
0	.91	.20	.20	.20	.20	.20	.20	.20	.20	1.10
6	1.64	1.46	2.18	3.66	.83	.54	2.05	1.77	4.23	2.28
12	1.93	1.18	1.67	1.76	2.02	1.59	3.22	1.33	4.59	2.21
18	3.06	1.93	2.44	2.89	1.43	1.05	1.88	.60	1.88	3.89
24	1.64	.86	1.75	.67	2.83	1.40	1.81	1.27	3.26	3.47
30	2.61	1.54	2.42	2.92	2.03	1.46	3.45	1.39	1.70	2.20
36	4.78	1.59	.98	2.98	5.56	1.71	2.27	1.10	3.68	5.03
42	4.51	3.28	1.03	2.74	2.78	2.21	2.68	1.30	2.20	5.94[a]
48	2.55	.99	.70	3.03	3.06	1.84	3.74	1.31	3.89[a]	42.50[a]
52	2.52	1.13	1.08	1.82	3.55	1.16	3.20	.20	37.79[a]	10.61
56	5.92	2.19	1.76	2.32	5.89	2.08	3.48	.20	17.34	1.68
60	2.08	2.55	1.83	1.49	2.60	3.18	3.88	1.03	1.71	.85
64	1.88	9.00[a]	1.10	.91	15.88[a]	1.10	1.69	.81	.20	.20
68	1.54	34.58[a]	1.26	.80	33.94[a]	2.48	2.90	.37	.90	.37
72	1.63	7.85	1.14	2.98	4.62	7.03[a]	3.08	.43	.20	.72
76	3.35	.80	1.50	2.67	.79	35.81[a]	3.94	1.43	.94	.75
80	1.80	.20	2.01	1.86	.20	12.85	19.67[a]	.20	.54	-
84	1.54	.20	2.06	1.91	.74	.57	26.66[a]	.20	.85	1.05
88	.69	.20	.20	.20	.20	.20	1.34	.20	-	-
92	.75	.20	1.01	1.04	.20	.20	1.20	.20	-	-
96	.71	.20	.60	9.97[a]	.75	.20	.50	.20	.53	2.29
100	2.09	-	.65	34.86[a]	-	.20	1.60	2.59[a]	-	-
104	1.23	-	2.33[a]	7.04	-	-	.60	46.94[a]	-	-
108	1.82	-	43.92[a]	.82	1.43	.20	1.23	12.40	2.39	.64
112	2.17	-	6.79	.43	-	-	-	.47	-	-
116	1.25	-	.97	.20	-	-	-	.20	-	-
120	2.26	1.47	1.45	.20	2.09	.91	1.95	.20	2.99	3.28
124	8.49[a]	-	.20	.20	-	-	-	.20	-	-
128	29.41[a]	-	.20	-	-	-	-	.20	-	-
132	6.81	2.04	.20	.65	2.88	.20	1.64	.20	2.69	1.89
136	1.83	-	1.21	-	-	-	-	-	-	-
140	1.76	-	-	-	-	-	-	-	-	-
144	1.25	.84	.92	1.39	1.63	.50	2.81	.20	2.17	1.64

[a]Time of LH peak

Table 9. Plasma prolactin (ng/ml) following $PGF_{2\alpha}$ injection.

Hours from	21.3 C					32.0 C				
$PGF_{2\alpha}$	ANIMAL NO. 63	68	87	89	94	62	64	86	88	92
-18	50.7	7.9	11.5	10.6	10.5	18.0	10.9	10.4	12.0	14.3
-12	15.1	14.0	9.1	10.0	13.5	15.3	13.9	16.5	25.9	15.4
0	5.5	9.6	5.8	7.9	9.3	14.0	12.0	17.1	21.3	12.8
6	8.5	5.9	8.4	6.9	10.8	19.9	10.6	12.0	32.4	13.5
12	5.0	10.3	18.1	14.3	12.4	33.9	16.6	15.1	35.9	17.5
18	18.5	13.5	14.5	13.6	18.9	22.9	13.4	13.4	25.6	13.0
24	7.3	5.6	12.1	12.0	14.0	15.1	11.6	12.6	21.9	10.5
30	8.9	7.0	10.8	17.5	15.9	20.8	13.9	13.1	22.6	17.4
36	39.1	13.1	33.5	14.1	18.4	14.6	14.4	18.0	20.8	14.4
42	15.5	13.0	9.6	14.3	15.3	12.3	13.1	6.6	23.8	9.9
48	10.6	3.3	8.9	19.6	12.9	13.4	13.6	10.0	21.0	9.9
52	11.5	6.9	12.9	16.5	11.9	17.1	13.4	9.0	19.3	8.0
56	15.1	11.6	14.0	14.4	23.8	11.8	7.4	10.7	25.8	13.0
60	15.5	8.3	18.3	13.3	15.3	13.3	15.0	17.4	27.5	15.5
64	18.9	13.8	24.4	11.8	12.9	13.6	13.3	11.0	33.0	15.1
68	30.0	10.6	17.9	10.3	14.1	10.8	12.0	11.8	29.5	12.0
72	16.5	14.4	16.8	13.0	19.3	19.6	14.5	16.3	24.5	12.4
76	12.0	9.5	15.1	12.3	15.3	16.5	16.8	12.3	13.5	14.5
80	10.9	5.9	13.3	5.9	5.6	17.5	13.6	13.6	17.7	-
84	42.3	8.9	29.5	19.9	14.9	15.4	11.3	7.4	19.6	14.3
88	14.1	12.6	22.0	23.0	12.1	16.5	9.4	9.6	-	-
92	34.1	16.4	31.9	26.9	43.5	10.3	15.8	12.9	-	-
96	9.3	18.9	12.1	12.9	8.0	13.4	7.0	7.5	33.1	9.4
100	14.0	-	11.5	8.9	-	13.1	12.3	10.2	-	-
104	9.9	-	8.0	13.1	-	-	7.6	9.5	-	-
108	24.1	-	14.4	16.0	15.1	13.4	12.0	13.1	20.6	11.6
112	23.0	-	19.0	13.9	-	-	-	9.6	-	-
116	32.8	-	15.4	9.6	-	-	-	16.2	-	-
120	12.9	10.5	14.8	10.0	12.0	8.5	4.6	14.1	12.5	9.0
124	18.3	-	14.0	12.5	-	-	-	12.6	-	-
128	12.5	-	11.1	-	-	-	-	13.3	-	-
132	15.6	11.6	13.8	7.8	10.9	11.9	7.1	8.0	24.3	9.0
136	11.4	-	33.3	-	-	-	-	-	-	-
140	13.1	-	-	-	-	-	-	-	-	-
144	5.9	8.5	6.5	10.8	5.0	6.5	7.5	6.9	17.8	4.6

Table 10. Plasma corticoids (ng/ml) following $PGF_{2\alpha}$ injection.

Hours from $PGF_{2\alpha}$	21.3 C					32.0 C				
	ANIMAL NO.									
	63	68	87	89	94	62	64	86	88	92
-18	11.0	11.2	14.2	14.0	15.9	6.5	8.5	3.1	10.1	6.1
-12	2.1	6.5	2.7	1.4	4.1	6.6	15.9	4.8	7.7	6.6
0	6.1	10.4	13.2	15.4	6.8	9.1	10.3	18.5	16.8	12.7
6	5.4	17.0	11.3	5.4	4.3	11.7	12.5	5.9	4.4	10.4
12	13.1	11.1	4.5	4.7	5.1	14.5	12.2	3.2	1.8	3.5
18	2.7	8.3	15.3	17.5	2.3	8.2	6.6	5.2	8.5	8.5
24	4.4	3.2	3.8	9.5	2.5	9.2	3.1	4.6	6.9	3.3
30	4.2	13.7	8.6	7.4	4.9	14.3	8.8	14.7	2.8	10.8
36	5.3	4.5	13.8	2.4	8.4	10.9	9.3	10.4	12.5	3.6
42	2.3	9.3	7.7	14.5	5.5	13.9	16.2	3.8	13.1	4.4
48	1.8	3.4	8.0	3.2	12.2	11.0	6.4	5.4	24.3	3.6
52	2.8	7.3	8.0	.7	9.9	16.5	13.5	7.7	3.6	6.9
56	12.0	12.8	18.9	8.0	12.9	4.1	11.0	8.4	6.0	5.6
60	4.9	8.1	11.1	7.8	9.9	8.5	13.4	12.5	9.8	2.2
64	3.2	4.0	3.4	3.4	6.4	10.7	15.4	7.6	10.5	9.6
68	7.1	5.2	14.1	2.8	6.1	17.7	10.5	4.2	6.1	3.1
72	1.5	8.5	10.4	1.4	1.2	7.4	9.0	11.0	3.4	1.6
76	3.6	3.7	3.6	13.2	2.2	5.4	3.8	3.9	4.0	3.1
80	2.0	2.3	2.8	8.3	5.7	7.3	6.7	5.3	3.1	-
84	8.0	5.2	7.1	9.1	7.1	12.6	5.7	1.0	1.9	3.7
88	1.7	2.3	13.6	10.3	2.9	3.4	6.5	3.0	-	-
92	34.8	13.4	6.9	41.7	26.0	8.1	7.6	11.3	-	-
96	2.5	6.6	9.2	8.1	4.5	3.9	5.5	10.1	5.3	4.6
100	6.7	-	8.7	13.1	-	10.3	2.9	13.8	-	-
104	13.6	-	19.0	9.1	-	-	8.7	6.7	-	-
108	14.8	-	21.5	8.9	11.9	7.1	8.1	9.4	4.9	5.5
112	1.6	-	2.5	7.6	-	-	-	8.7	-	-
116	11.8	-	10.8	1.1	-	-	-	9.3	-	-
120	3.4	2.8	9.1	7.4	5.4	4.0	5.3	11.5	3.8	8.9
124	10.0	-	22.8	9.1	-	-	-	12.1	-	-
128	4.9	-	3.5	-	-	-	-	4.0	-	-
132	4.4	1.4	2.5	2.1	2.7	3.6	8.1	2.1	7.8	2.9
136	14.2	-	13.4	-	-	-	-	-	-	-
140	4.5	-	-	-	-	-	-	-	-	-
144	1.7	11.6	9.1	6.1	2.8	3.5	9.8	7.9	8.6	7.9

Table 11. Plasma corticoids (ng/ml) prior to and following 200 IU ACTH.

HOURS FROM ACTH INJECTION	21.3 C ANIMAL NO.					32.0 C ANIMAL NO.				
	63	68	87	89	94	62	64	86	88	92
-2	8.0	19.8	9.5	14.8	17.3	9.8	6.7	9.6	13.6	18.6
-1	16.9	6.2	4.6	25.5	9.3	15.1	12.0	12.2	10.3	8.2
0	4.8	3.9	4.1	3.6	1.8	7.2	4.0	2.9	2.9	20.6
.25	55.9	41.7	34.0	62.0	58.4	-	41.8	87.3	41.7	97.8
.50	74.8	62.3	117.8	42.5	35.6	81.4	77.6	68.9	54.8	76.8
.75	123.8	100.2	111.7	100.5	39.8	62.2	42.4	45.3	42.5	75.4
1	49.2	26.9	137.4	89.3	66.6	83.0	49.8	29.6	110.6	71.8
2	48.3	92.7	72.8	64.7	168.0	62.2	36.8	95.4	54.0	84.9
3	35.7	119.7	77.9	64.9	140.9	35.7	48.5	66.0	50.3	16.4
4	39.5	12.4	29.4	22.3	41.5	36.9	43.3	39.9	35.6	23.7
5	41.4	11.9	31.7	16.3	69.8	21.3	16.6	35.7	16.0	32.1
6	12.8	9.2	20.8	10.6	25.1	9.3	23.5	26.9	46.3	13.1
7	8.9	8.9	18.5	15.0	22.5	16.8	15.1	14.7	18.6	16.9
8	5.9	3.3	7.7	4.0	3.2	11.7	9.7	13.9	10.8	9.5
9	3.7	3.3	6.3	4.3	15.0	9.9	8.2	13.5	4.0	11.4
10	4.4	4.1	4.5	4.8	5.9	3.5	7.2	8.6	5.5	3.7
11	4.9	3.6	5.8	5.9	5.2	2.4	4.1	3.8	4.8	4.0
12	3.8	4.3	1.5	5.1	14.3	2.7	3.9	3.2	5.8	4.3

Table 12. Plasma progestins (ng/ml) prior to ACTH injection.

HOURS FROM ACTH INJECTION	21.3 C					32.0 C				
	ANIMAL NO.									
	63	68	87[a]	89	94[a]	62	64	86	88	92
-2	2.16	2.96	1.83	2.65	3.08	1.53	1.76	3.08	2.40	1.42
-1	4.00	2.00	2.33	2.80	2.69	1.87	1.85	.99	1.37	1.87
0	5.31	2.06	2.02	2.98	2.32	2.08	1.40	1.69	3.51	3.92

[a]Pregnant

Table 13. Simple correlations between hormones and temperatures.

	LH	Air Temperature	ΔT_{u-a}	Aortic Temperature	Uterine Temperature
Estradiol[a]	.16	-.24	.02	.23	.23
LH[b]		.11	-.11	-.03	-.06
Air temperature[b]			-.38**	.15	.05
ΔT_{u-a}[b]				-.08	.17
Aortic temperature[b]					.97**

[a] $n=34$; [b] $n=49$

**($P<.01$)

Table 14. Analysis of variance for aortic and uterine temperatures.

Source	d.f.	Aortic Temperature MS	Uterine Temperature MS
Cow	1	3.69**	1.79**
Time of Day	1	.01	.12
Time of Day (Q)	1	2.61**	3.20**
Error	45	.17	.21

**(P<.01)

Table 15. Hormonal and temperature measurements for G665 (G) and JN15 (J).

Time following $PGF_{2\alpha}$ (hr.)	LH (ng/ml)		Estradiol (pg/ml)		ΔTu-a (C)		Taorta (C)		Tuterus (C)		Tair
	G	J	G	J	G	J	G	J	G	J	Barn (C)
0	.2	.2	19.0	13.8	.54	.40	39.25	38.44	39.79	38.84	27.26
6	.2	.2	13.3	17.1	.52	.44	39.27	38.83	39.79	39.27	27.28
12	.4	.7	-	7.7	.59	.44	39.08	38.84	39.67	39.28	25.34
18	.3	.2	-	7.2	.58	.49	38.63	38.94	39.21	39.43	25.58
24	.2	.2	-	-	.44	.31	38.45	39.21	38.88	39.52	30.61
30	.4	.3	17.0	7.2	.54	.33	38.46	39.39	39.01	39.72	28.11
36	.3	.2	-	20.1	.53	.31	38.53	39.36	39.06	39.67	27.16
42	.2	.6	13.0	-	.54	.23	38.26	38.61	38.80	38.84	26.63
46	.5	.2	22.3	-	.44	.28	38.66	39.14	39.10	39.42	30.63
50	.7	.2	7.7	9.7	.43	.30	39.28	39.50	39.72	39.80	32.51
54	.7	.6	-	26.1	.55	.36	39.88	39.90	40.42	40.26	30.16
58	.2	.2	24.6	12.5	.64	.33	39.68	39.84	40.32	40.16	27.19
62	.3	.2	14.3	-	.65	.36	38.79	39.41	39.43	39.77	26.20
66	.5	.2	12.6	10.1	.55	.30	38.19	38.74	38.75	39.05	25.71
70	5.5	.4	15.9	-	.26	.25	38.21	39.13	38.47	39.38	30.85
74	30.3	.5	10.2	-	.34	.24	38.60	39.26	38.94	39.50	31.82
78	6.0	.5	7.8	18.9	.44	.27	39.00	38.94	39.44	39.21	31.00
82	.8	1.3	-	12.9	.69	.35	39.19	39.73	39.88	40.08	27.27
86	.2	22.7	8.3	26.7	.73	.47	38.79	39.75	39.52	40.22	26.37
90	.2	1.8	-	22.1	.63	.51	38.27	40.00	38.91	40.50	26.66
94	.2	.2	5.3	9.9	.48	.27	38.27	39.25	38.76	39.51	30.37
98	.2	.2	3.2	5.9	.57	.33	38.81	39.79	39.38	40.12	32.30
102	.2	.2	12.7	-	.65	.49	39.23	40.01	39.88	40.50	31.51
106	-	.2	-	6.6	-	.48	-	39.88	-	40.36	28.87
110	-	.2	-	11.7	-	.56	-	39.65	-	40.21	28.33
114	-	.2	-	-	-	.55	-	39.98	-	40.53	28.11

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The author married Judy Keller in 1971, and they have a daughter, Jennifer and a son, James. He is a member of Gamma Sigma Delta, Alpha Zeta, Sigma Xi, the American Dairy Science Association, the American Society of Animal Science and the Society for the Study of Reproduction.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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